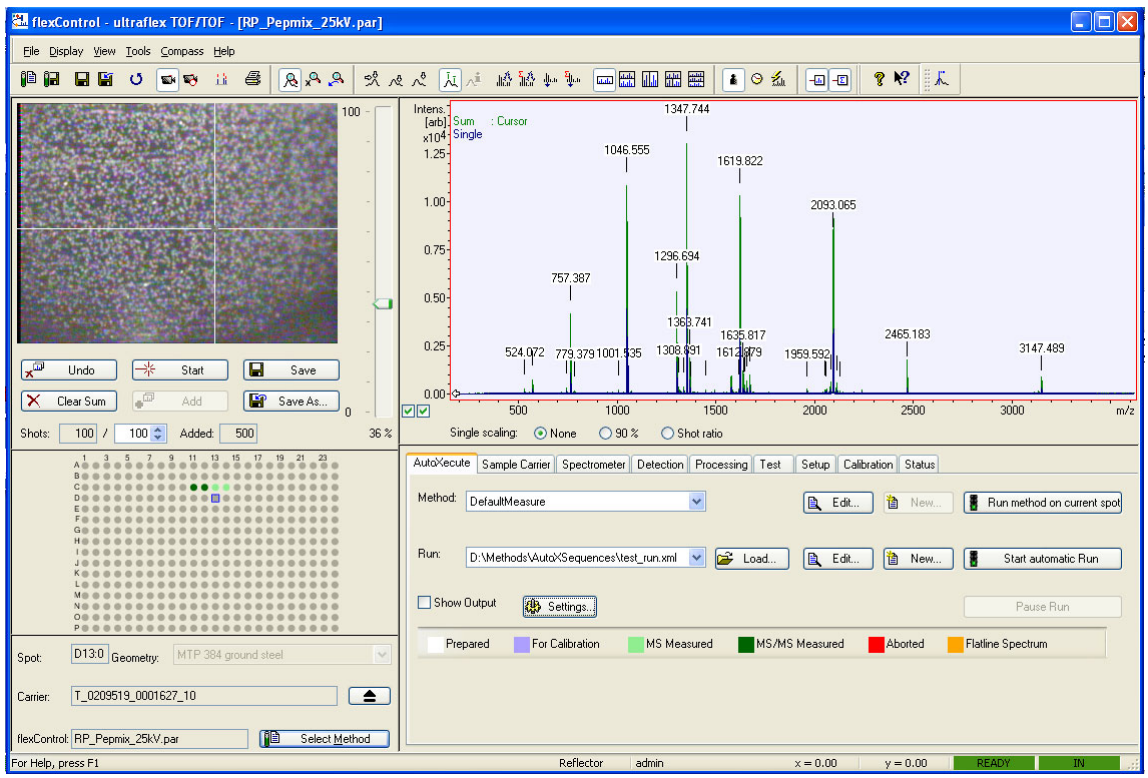
flexControl

User Manual



Version 3.0

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How to Obtain Support

If you encounter problems with your system please contact a Bruker representative in your area, or:

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28359 Bremen
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Phone: +49(421) 2205-430
Fax: +49(421) 2205-370
Email: maldi.support@bdal.de

software support
Phone: +49(421) 2205-345
Fax: +49(421) 2205-390
Email: maldi.sw.support@bdal.de

application support
Phone: +49(421) 2205-184
Fax: +49(421) 2205-104
Email: maldi.appl.support@bdal.de

1 INTRODUCTION

flexControl is a program designed to configure and operate Time Of Flight mass spectrometers of the Bruker flex-series, such as the **microflex, autoflex, ultraflex, Biflex and Reflex**. This program runs under Microsoft® Windows® 2000 (SP4) or XP® (SP2) operating systems. The Bruker post-processing software **flexAnalysis** can reload recorded spectra from the disk for post processing.

Before installing **flexControl 3.0** for the first time, the program **Microsoft .NET Framework 1.1** must have been installed, because **flexControl** (since the release 2.2) is based on features, which **Microsoft .NET Framework 1.1** provides. On most XP systems and if a further "Compass for flex version" is running, **Microsoft .Net** is already installed. For more information have a look into the Installation Instructions that are part of the **Compass** installation.

Figure 1 Dialog on the Compass Installation CD

flexControl uses standard IBM-PC and Microsoft® Windows® conventions to operate with windows, menus, dialog boxes, and the mouse. The operator is supposed to be familiar with the basic operation of the computer and with Microsoft® Windows® software. Some general instructions are given here, but if additional assistance is necessary regarding the basic use of a computer, please review its accompanying documentation.

Depending on the mass spectrometer (**ultraflex TOF/TOF**, **autoflex**, etc.), **flexControl** offers features specific to the respective unit.

flexControl 3.0 (Compass 1.2 for flex) is able to operate in combination with the Bruker **Compass Security Pack**. The Bruker **Compass Security Pack** and user management are described in a separate manual.

Main features of the Bruker **Compass Security Pack** are electronic signatures, audit trailing, and a user management. This is different to the user management of the operating system! The Bruker User Management is a tool that allows managing the access to Bruker applications and assigning individual privileges to users. Operator IDs and passwords identify such a privilege. Depending on these privileges the access of a user to features of Bruker applications is more or less restricted.

On saving data files, the operator can electronically sign them, if he has got this specific privilege. Electronic signatures consist of the operator ID and his password.

Audit trails are useful to trace back the operations performed in a specific moment.

2 GETTING STARTED

2.1 Installation

Please refer to the separate installation instructions on the installation CD-ROM, or contact Bruker via e-mail if you want to install a **flexControl 3.0** on your computer.

2.2 Starting flexControl

Due to Windows conventions **flexControl** can be started via the **Windows Start** menu. During installation of the Bruker Daltonics Applications package, a **Bruker Daltonics** folder was automatically created in the Start menu's **Programs** folder, which contains the respective applications. A second way to launch **flexControl** is double clicking the corresponding icon on the Windows desktop.

During start up of the program (Figure 3) the dialog shown in Figure 12 automatically opens to choose a method. **flexControl** may acquire four different kinds spectra and therefore offers four different method types, identified by specific extensions:

- <file name>.par (for standard applications).
- <file name>.lft (for LIFT applications on a tandem mass spectrometer).
- <file name>.psm (for FAST applications).
- <file name>.isd (for applications with undigested proteins).

In case a LIFT method is loaded the LIFT page (section 3.4.9.10) appears additionally and the Calibration page (section 3.4.9.9) changes. If a FAST method is loaded the FAST page is shown (section 3.4.9.11). If a LIFT method is loaded on a non-LIFT instrument the following message appears:

Figure 2 flexControl does not have detected a tandem instrument

2.3 Closing flexControl

Click the application's “Close” button or from the **File** Menu select **`Exit`**, or press **`Alt + F4`**. If the currently loaded method or instruments setting file (*.isset) have been changed but not saved message dialogs appear and ask for saving. If you are not sure you may select “No” or “Cancel” to stay in **flexControl** and save the files with other file names. Afterwards it is possible to close **flexControl** without problems.

3 GRAPHICAL USER INTERFACE (GUI)

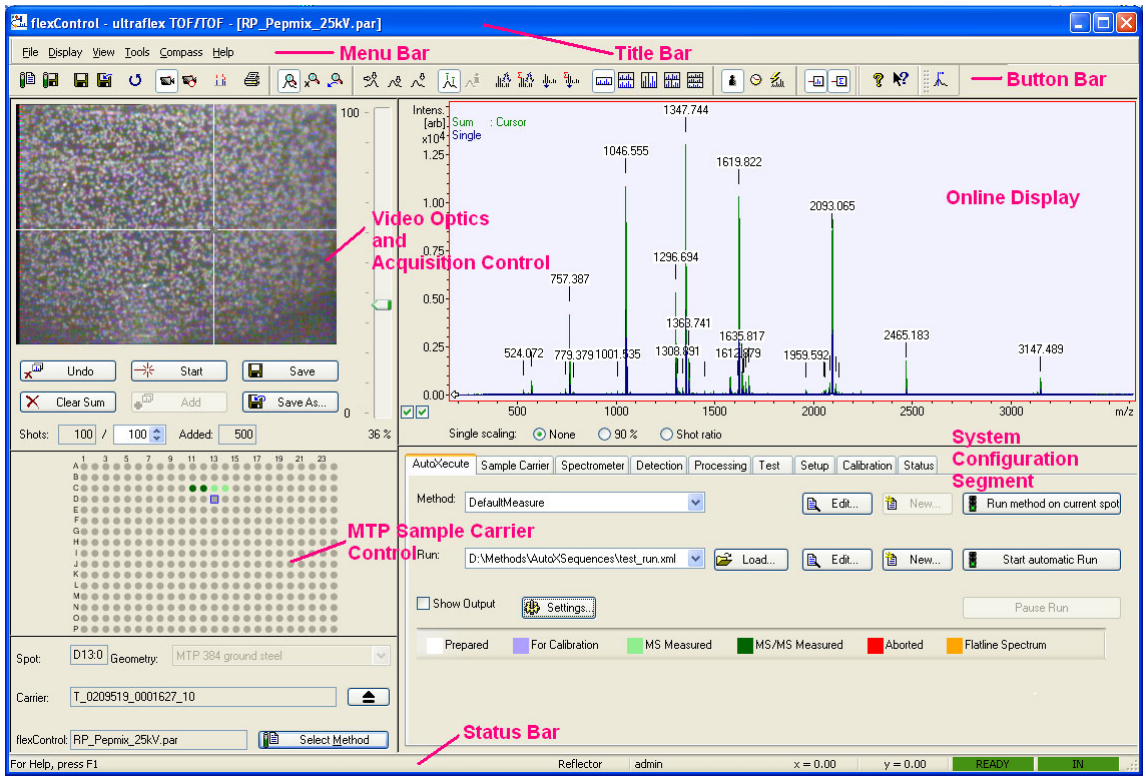


Figure 3 Graphical User Interface of flexControl

The GUI shows the current entire status of the system. It is used to configure and control a mass spectrometer of the flex-series in order to acquire data.

The GUI (Figure 3) is composed of the following items:

- Title Bar (section 3.1)
- Menu Bar (section 3.4)
- Tool Bar (section 3.2)
- Status Bar (section 3.4.3.3)
- Video Optics and Acquisition Control (3.4.7.1)
- MTP Sample Carrier Control (section 3.4.7.2)
- Online Display (section 3.4.8)
- System Configuration Segment (section 3.4.9).

3.1 Title Bar

The **Title Bar** (Figure 4) reflects the type of the mass spectrometer, e.g., an ultraflex and the currently loaded method. The left end of the **Title Bar** contains the application's “Control-menu” button and the right end contains the “Minimize”-, “Maximize”-, and “Close”- buttons corresponding to all other Windows applications.

Figure 4 Example of a Title Bar

3.2 Toolbar

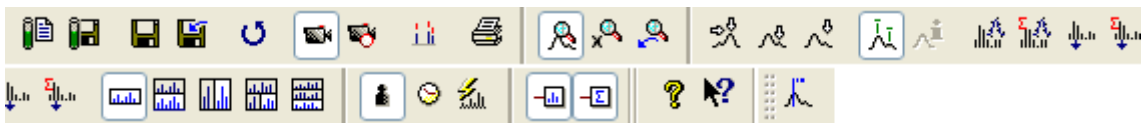


Figure 5 Toolbar of flexControl

Feature buttons have been given for commands that are regularly used. These buttons are organized into five groups.

- Buttons referring to the File Menu (section 3.4.1).
- Buttons referring to the Display Menu (section 3.4.2).
- Buttons referring to the View Menu (section 3.4.3).
- Buttons referring to Tools Menu (section 3.4.4).
- Buttons referring to the Help Menu (section 3.4.5).

To configure the **Toolbar**, i.e. add or remove icons, please refer to section 3.4.3.2.

3.3 Status Bar

The **Status Bar** (Figure 6) is located at the bottom of the GUI. It contains several data like as instrument mode, current user, coordinates of the mouse pointer and state of the instrument and target location.

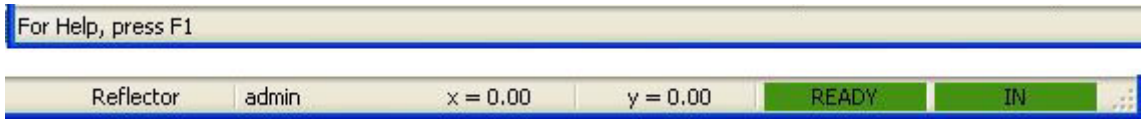


Figure 6 Status Bar of flexControl

3.4 Menu Bar

The **Menu Bar** (Figure 7) consists of several pull down menus. Each menu contains groups of commands related to this menu. Many menu features can also be used with feature buttons (section 3.2) or by executing the corresponding short cuts.

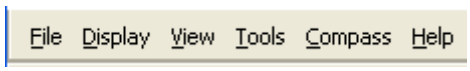


Figure 7 Content of the Menu Bar

3.4.1 File Menu

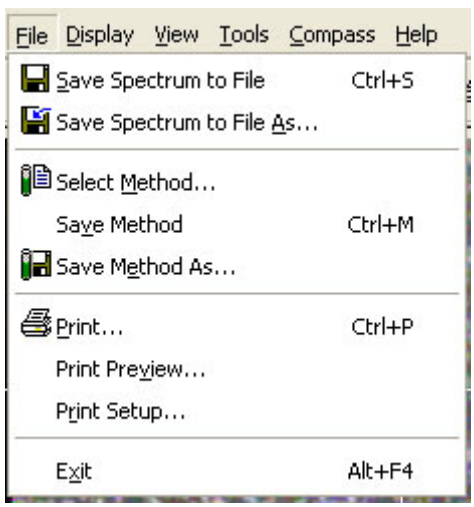


Figure 8 Contents of the File Menu

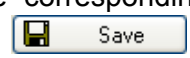
The **File** Menu (Figure 8) is composed of commands to handle methods, save data, and test and do print-outs.

3.4.1.1 Overview

Table 1 Feature buttons of the File Menu with corresponding tasks

Feature button	File Menu commands	Shortcut	Description
	Save Spectrum to file	Ctrl + S	Saves spectra.
	Save Spectrum to file As		Saves spectra with another name.
	Select Method		Opens a dialog box to select a method from the file system.
	Save Method		Saves the method.
	Save Method As		Opens a dialog to save a method with a new name.
	Print	Ctrl + P	Prints a report of the selected analyses.
	Print Preview		Displays print preview using the selected report layout.
	Print Setup		Displays the Windows dialog for printer setup.
	Exit		Closes **flexControl**.

3.4.1.2 Save Spectrum to File

The **`Save Spectrum to File`** command, the corresponding icon of the **Toolbar**, the short cut (**`Ctrl + S`**) and the button 

in the **Acquisition Control** section (section 3.4.7.1) are used to save the current spectrum to disk.

flexControl saves spectra using the XMASS-file format. An example is shown in Figure 9. **flexControl** handles names of saved spectra in a manner that overwriting is automatically excluded. Every save-process in the same directory generates a numerated subfolder with a new analysis tree.

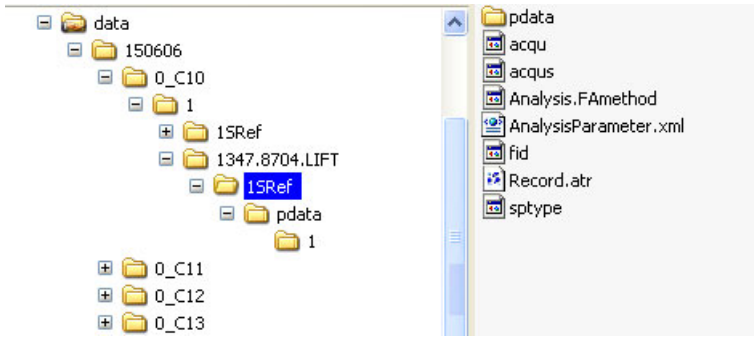


Figure 9 Example of the XMASS file structure

Raw data are write-protected. They are stored in a **fid**-file. Already processed data are stored in a **1r**-file. Each analysis contains both files. If raw data are not yet processed both the **fid** and the **1r**-file are the same.

Saving a file the first time, the XMASS file structure has to be generated, and the dialog shown in Figure 10 appears. If the “Save” command has already been used in a **flexControl** session for this spectrum type, the saving is done without any dialog, since a valid path- and sample name exist.

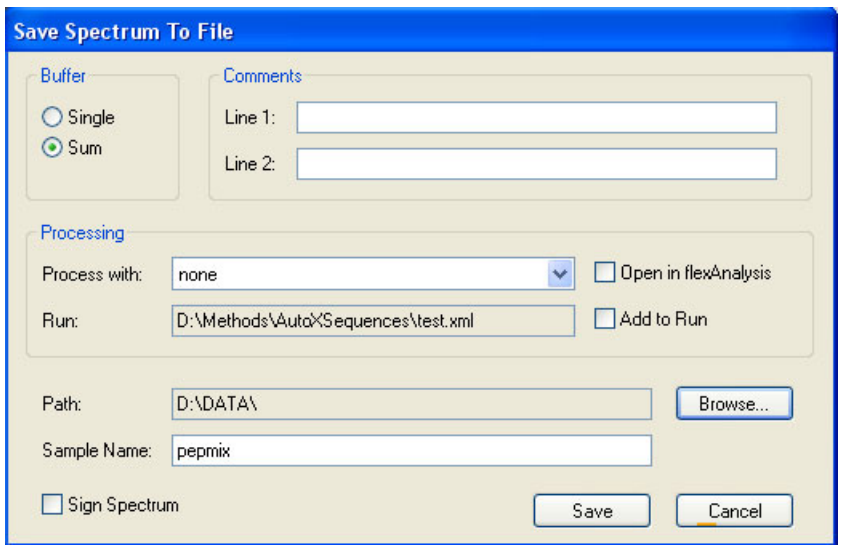


Figure 10 Save (As) dialog to specify data directory and possible post-processings

- **Single/Sum**: The radio buttons decide if the single or the sum buffer is saved.
- **Comments**: Enter some comments that are saved with the spectrum and appear in post-processing tools like flexAnalysis and BioTools.

- **Processing with**: Specifies a method for post processing in flexAnalysis if it is installed on the same computer. When opening a spectrum in flexAnalysis the assigned processing method will be executed.

- **Open in flexAnalysis**: If this checkbox is activated, the spectrum is automatically sent to flexAnalysis. A possible assigned method is executed. If no method is assigned, the spectrum opens in flexAnalysis with a defined default method (see flexAnalysis manual).

- **Run**: This feature should be used if you want to save the information of a measured MS spot (path, sample name and comments) to use them for MS/MS measurement on this spot afterwards. With activated **Add to Run** checkbox (see below) the MS information is stored in the displayed AutoXecute **Run**. It is possible to transfer (different) comments and sample names for different spots in one run. During the following LIFT measurement the spot information is automatically loaded into the Save (As…) dialog to be able to save LIFT spectra next to the belonging MS spectrum. To use this feature correctly

    1. Create and load an AutoXecute run in the AutoXecute tab (section 3.4.9.1.3). This should contain comments, path and sample name at least for one position, but you also can define the settings for all the spots you want to measure manually.

    2. Acquire an MS spectrum, open the “Save As…” dialog and set the checkbox **Add to Run**. If available, the information for the spot are read in from the run (see below), otherwise the dialog will be empty or fulfilled with the latest information.

    3. Change the settings if necessary and save the spectrum

    4. Load a LIFT method and start the manual LIFT acquisition on the green spot where the MS spectrum has been acquired.

    5. Open the Save As dialog: the correct spot information have been transferred and the LIFT spectrum can be saved directly.

- **Add to Run**: If a run is shown and this checkbox is activated, the data directory, sample name information, comments and as well a possibly selected flexAnalysis method are transferred if they are available in the run and transferred back during the save process to the run.

- **Path**: Select a data directory for the results with the

button to change the path.

- **Sample**: Type in a sample name.

- **Sign Spectrum**: If the Compass Security Pack is installed, spectra can be signed during the save process. The user has to have the specific privilege to sign. Operator and Password additionally are necessary. **Note**: If a spectrum is signed in **flexControl**, it is write-protected. No post processing results can be stored with **flexAnalysis** or any other programs.

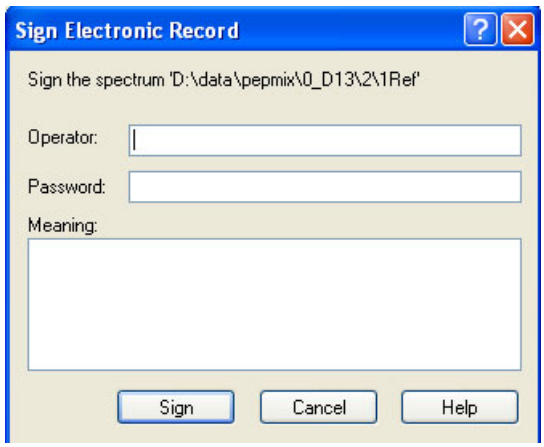
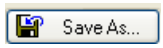


Figure 11 Sign a spectrum

3.4.1.3 Save Spectrum to File As

The **`Save Spectrum to File As`** command, the corresponding icon of the **Toolbar**, and the button 

on the Video Optics and Acquisition Control 3.4.7.1) are used to save the current spectrum to disk with another name. The dialog is the same as for the **`Save Spectrum`** command. The difference is that it appears every time **`Save Spectrum to File As`** is executed.

This command is not accessible in case of a FAST measurement, as saving is automatically done after switching to the next segment. Even at the end of a manual FAST measurement the spectrum is automatically saved to the path and sample name selected on the FAST tab (section 3.4.9.11).

3.4.1.4 Select Method

The **`Select Method`** command and the corresponding icon on the **Toolbar** open a dialog to select a **flexControl** method:

- *.par for fingerprint spectra.
- *.psm for FAST measurements.
- *.lft for LIFT measurements in case of a tandem mass spectrometer.
- *.isd (**I**on **S**ource **D**ecay) for measurements of fragments arising inside the ion source.

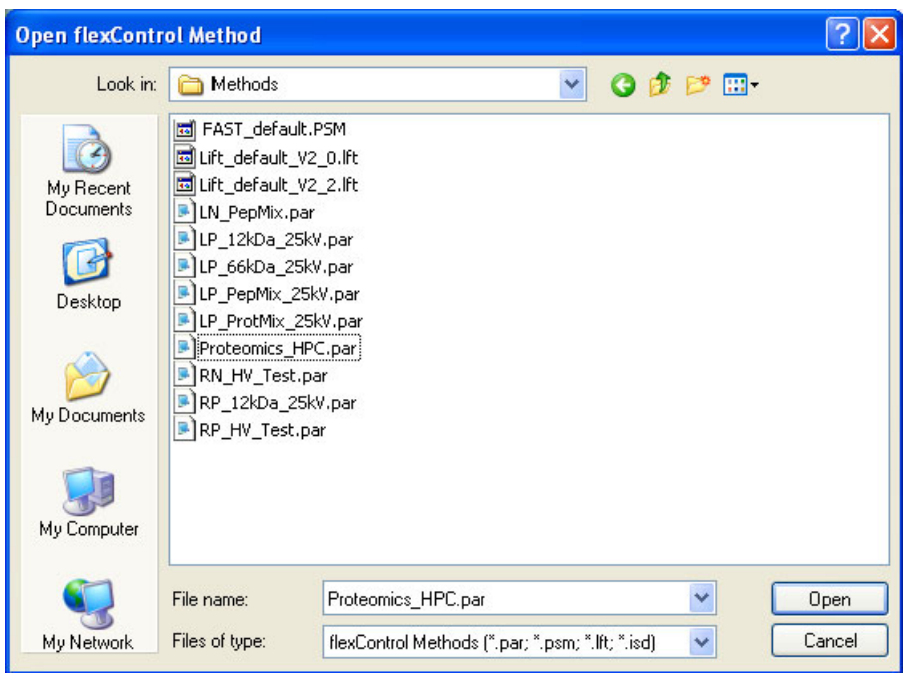


Figure 12 Open flexControl Method dialog

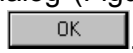
3.4.1.5 Save Method

The **`Save Method`** command saves the currently loaded (not write protected) method without any message.

3.4.1.6 Save Method As

The **`Save Method As`** command, and the corresponding icon of the **Toolbar** open a dialog to save the currently loaded method with a new name.

3.4.1.7 Print

The **`Print`** Command, the corresponding icon of the **Toolbar** and the short cut (**`Ctrl + P`**) open the print dialog (Figure 13) to select a printer. A printout can be made from here with the button  . Refer to section 3.4.1.8 to look at the report layout.

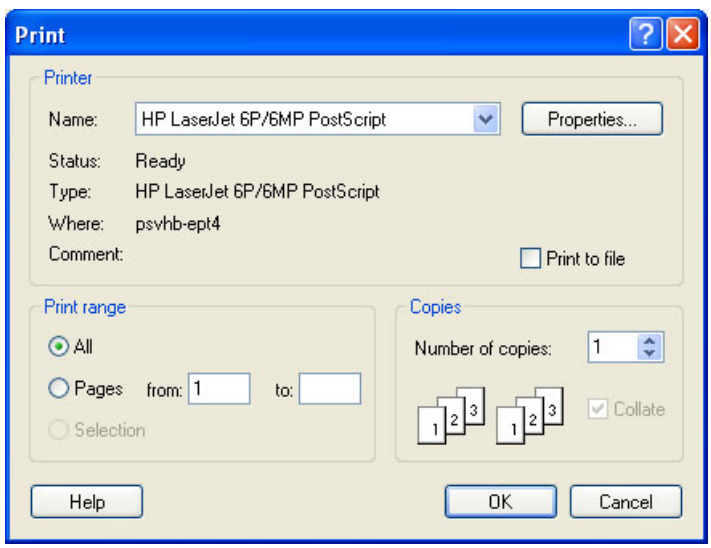


Figure 13 Print dialog

3.4.1.8 Print Preview

The **`Print Preview`** command is used to verify how the spectrum looks like prior to printing. The dialog shown in Figure 14 appears. A printout can be done from here with a click on the button “Print” in the upper left corner.

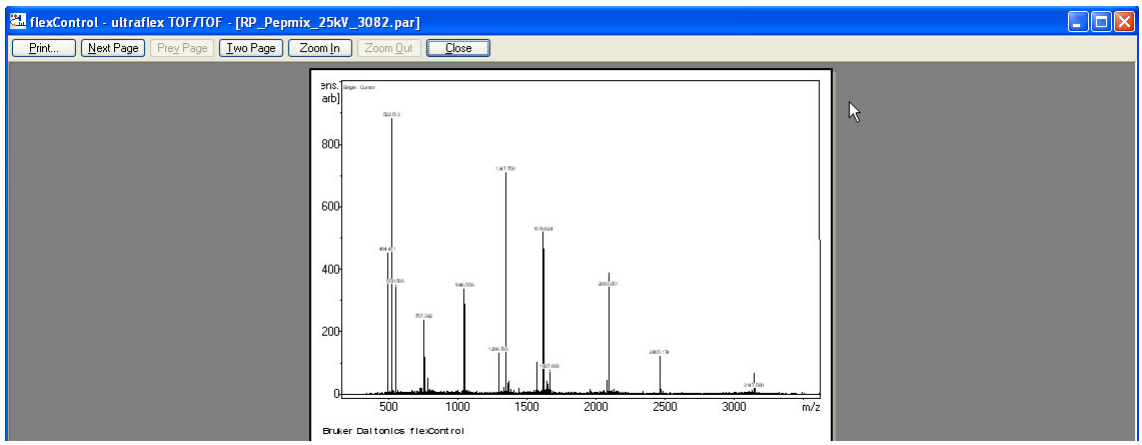


Figure 14 Print Preview dialog

3.4.1.9 Print Setup

The **`Print Setup`** command is used to adjust the printer. It opens the following dialog.

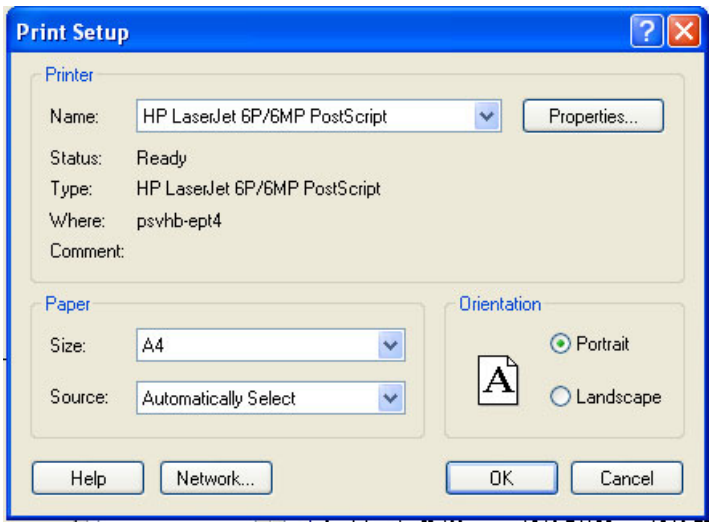


Figure 15 Print Setup dialog

3.4.1.10 Exit

The **`Exit`** command and the short cut (**`Alt + F4`**) close **flexControl**. The dialog shown in Figure 16 appears to be acknowledged.

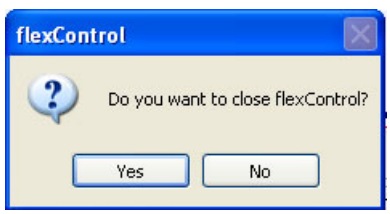


Figure 16 Close flexControl by applying “Yes”

Note: If you have meanwhile changed methods or instrument settings, corresponding messages may appear to save the changes (Figure 17, Figure 18). When you are not sure if important changes have been done you may select “No” or “Cancel” to stay in **flexControl** and save the files with other file names. Afterwards it is possible to close **flexControl** without further messages.

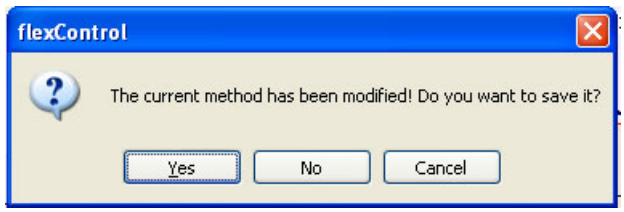


Figure 17 Question to save changed methods

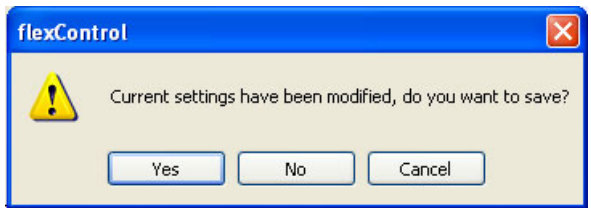


Figure 18 Question to save modified instrument settings

3.4.2 Display Menu

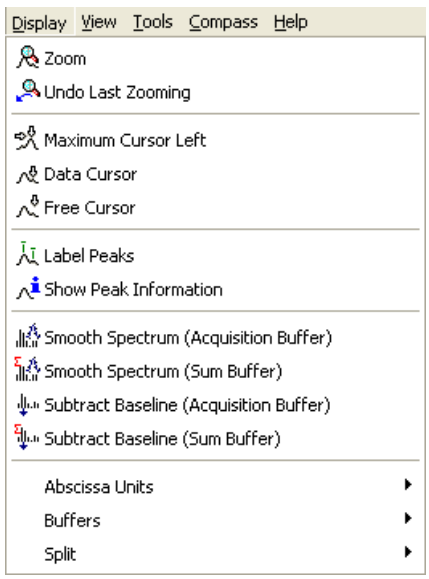


Figure 19 Contents of the Display Menu

The **Display** Menu (Figure 19) is composed of commands to manipulate spectra.

3.4.2.1 Overview

Table 2 Features of the Display Menu

Feature button	Display Menu commands	Shortcut	Description
	Zoom		Zooms the selected data range.
	Undo Last Zooming		Undoes the last zooming step.
	Maximum Cursor Left		Left click of the mouse button fixes this additional cursor to that y-position (top of a peak) next to the mouse pointer, which is just higher.
	Data Cursor		Accompanies vis-à-vis the arrow pointer of the mouse within the **Mass Spectrum Window**.
	Free Cursor		
	Label Peaks		
	Show peak information		
	Smooth Spectrum (Acquisition Buffer)		Smoothes the envelope of the spectrum in the active acquisition buffer.
	Smooth Spectrum (Sum Buffer)		Smoothes the envelope of the spectrum in the active sum buffer.
	Subtract Baseline (Acquisition Buffer)		Pulls down the spectrum of the active acquisition buffer to the baseline.
	Subtract Baseline (Sum Buffer)		Pulls down the spectrum of the active sum buffer to the baseline.
	Abscissa Units Mass		Mainly used for standard operation. **m/z** scale represents a calibrated mass spectrum.
	Abscissa Units Times		Mainly used for debugging purposes. **t [ns]** denotes a raw time-of-flight-spectrum displayed in nanoseconds.

Feature button	Display Menu commands	Shortcut	Description
	Abscissa Units Points		Mainly used for debugging purposes. Shows how many data points are distributed over the peak shape.
	Linear Detector Single Spectrum		Displays a single shot, usually the last acquired sequence of shots.
	Sum Spectrum		Displays at least a single shot, usually the last acquired sequence of shots.
	One Display		One display is shown.
	Two Displays		Two displays, one upon other.
	Two Displays		Two displays next to each other.
	Three Displays		Three displays.
	Three Displays		Three displays, one upon the other.

3.4.2.2 Zoom

The **Zoom** command and the corresponding icon of the **Toolbar** are used to enlarge a section within the **Mass Spectrum Window**. The **Zoom** function is active after the corresponding tool button is applied. Then the mouse pointer converts into the **Zoom** pointer (within the **Mass Spectrum Window**).

To enlarge a mass range, move the **Zoom** pointer to the initial position and press the left mouse button. Hold the button, move to the final position (in x and y direction), and release the mouse button. This action draws a rectangle inside the **Mass Spectrum Window**. The content will be shown enlarged.

3.4.2.3 Undo Last Zooming

The **Undo Zooming** command and the corresponding icon of the **Toolbar** are used to reverse the last zooming step that was performed in the **Mass Spectrum Window** via the **Zoom** command.

3.4.2.4 Maximum Cursor Left

The **Maximum Cursor Left** command and the corresponding icon of the **Toolbar** are used to create an additional cursor, which is always located left-hand to the mouse pointer. Left click of the mouse button fixes the cursor to that y-position (top of a peak) next to the mouse pointer, which is just higher.

3.4.2.5 Data Cursor

The **Data Cursor** command and the corresponding icon of the **Toolbar** are used to create an additional cursor that follows always the mouse pointer on the left side sliding over the peaks.

3.4.2.6 Free Cursor

The **Free Cursor** command and the corresponding icon of the **Toolbar** are used to create an additional cursor that accompanies the mouse pointer with an **F**. This mark might be fixed as desired by using the left mouse button.

3.4.2.7 Label Peaks

The **Label Peaks** command and the corresponding icon of the **Toolbar** are used to perform peak picking in the active buffer. The peak picking parameters can be set using the Processing Parameter dialog (Figure 75).

3.4.2.8 Show Peak Information

The **Show Peak Information** command, or the corresponding icon of the **Toolbar** are used to open a window (Figure 20), which contains the four (five) most important peak characteristics. This command is only available together with the activated **Maximum Cursor Left** button .

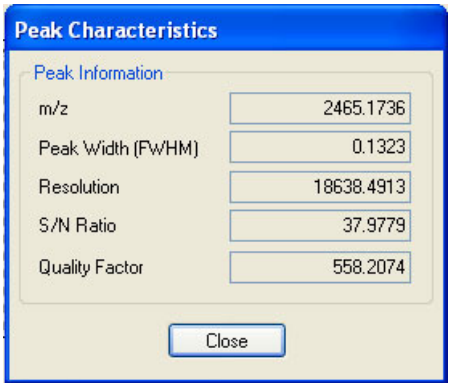


Figure 20 Example of peak characteristics

3.4.2.9 Smooth Spectrum (Acquisition/Sum Buffer)

The **`Smooth Spectrum (Acquisition/Sum Buffer)`** command and the corresponding icons / of the **Toolbar** are used to smooth the spectrum in the respective buffer. The algorithm used for this can be checked via the Processing Parameter method (section 3.4.9.5).

3.4.2.10 Subtract Baseline (Acquisition/Sum Buffer)

The **`Subtract Baseline (Acquisition/Sum Buffer)`** command, or the corresponding icon / of the **Toolbar** are used to subtract the baseline of the spectrum in the respective buffer. The algorithm used for this can be checked via the Processing Parameter method (section 3.4.9.5).

3.4.2.11 Abscissa Units

flexControl allows to choose between three scaling factors for the x-axis. The **`Abscissa Units`** commands and the respective icons of the **Toolbar** are used to assign one of these to the abscissa.

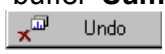
`Mass` : Mainly used for standard operations. **m/z** scale is default.

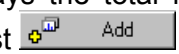
`Time` : Mainly used for debugging purposes. **t [ns]** denotes a raw time-of flight-spectrum displayed in nanoseconds.

`Points` : Mainly used for debugging purposes. Shows how many data points are distributed over the peak shape.

3.4.2.12 Buffers

On the TOF-instruments of the flex series are two buffers available. One is used to store a single spectrum the other one is used to add spectra.

- The buffer **Single Spectrum** displays at least a single shot, usually the last acquired sequence of shots.
- The buffer **Sum Spectrum** displays the total number of added spectra. The 

button reverses the last 

operation.

flexControl allows for scaling the single buffers with regard to the respective sum buffer. The available three scaling modes have no influence on the buffer contents. They are exclusively used for displaying spectra in the RTD (**R**eal **T**ime **D**isplay) (**Mass Spectrum Window**).

flexControl performs only scaling when data are already stored on the sum buffer and the corresponding sum buffer is chosen. Toggling between the scale modes takes only effect, when an acquisition is running. Changing the mode before or after an acquisition has no influence on the display.

Below the RTD (Figure 21) is an arrangement of three radio buttons to choose a suitable scaling mode.

- **None:** No scaling of the single buffer is performed.
- **90%:** The scaling of a single buffer is performed in a manner that its most intensive peak has 90% of the height of the most intensive peak of the sum buffer. This feature benefits in being able to compare both peaks in the single buffer and in the respective sum buffer as well concerning position, shape and size.
- **Shot ratio:** Using this mode scaling of the single buffer is done referring to the total number of shots. After the shot sequence is finished the peak size of the single buffer correspond to that one of the sum buffer. In this mode the user can observe the increase of the peaks intensity. Thus he can verify immediately the current peak intensity and its deviation from the average.

3.4.2.13 Split

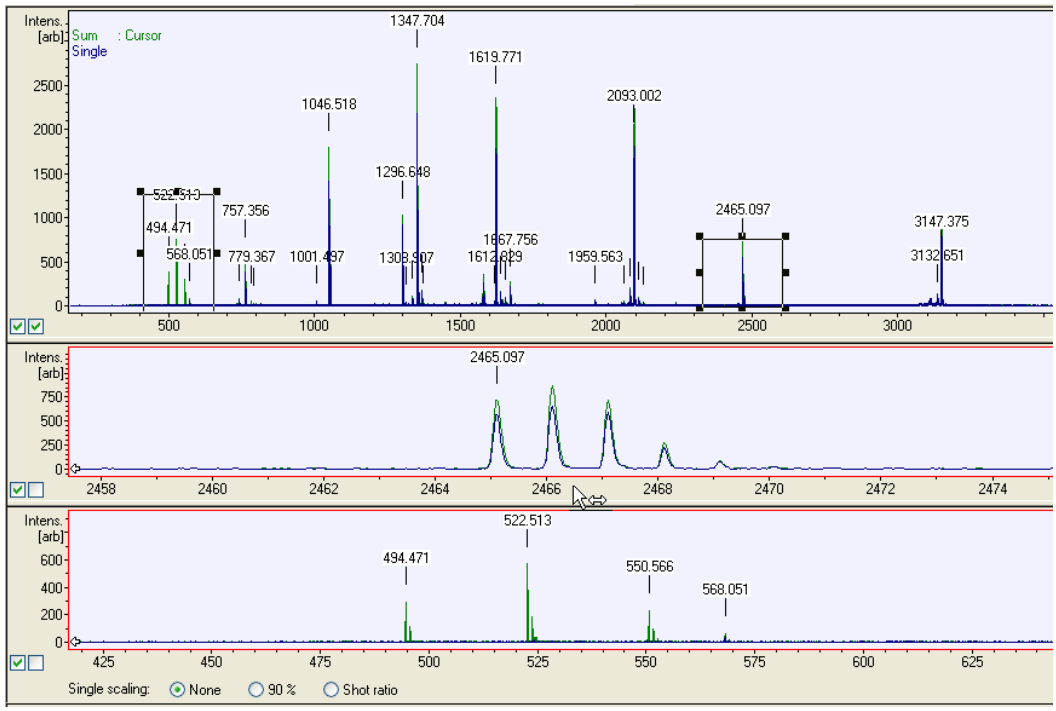


Figure 21 Mass Spectrum Window displayed in one of the split modes

The **`Split`** command, or one of the corresponding icons on the **Toolbar** are used to display more than one spectrum at a time (Figure 21), in order to facilitate manipulations, e.g., to compare segments of a spectrum.

3.4.3 View Menu

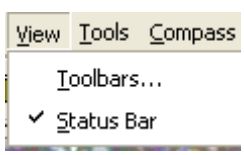


Figure 22 Contents of the View Menu

The **View** Menu (Figure 22) is composed of commands to configure the GUI.

3.4.3.1 Overview

Table 3 Features of the View menu

Feature button	View Menu commands	Shortcut	Description
	Toolbars		Opens the dialog toolbars.
	Status Bar		Show or hides the Status Bar.

3.4.3.2 Toolbars

The **`Toolbars`** command opens the **Customize Toolbar** dialog box (Figure 23) to customize the **Toolbar** of the GUI. Select an item in the left list of buttons and apply the “Add” button. Immediately it will be shifted to the right list and displayed on the **Toolbar**. To remove an item from the **Toolbar** select it in the right list of buttons and apply the “Remove” button.

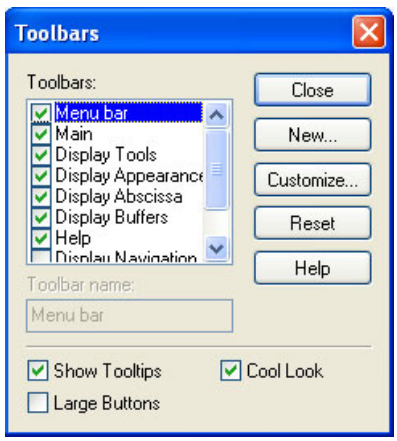


Figure 23 Toolbars dialog box

3.4.3.3 Status Bar

The **`Status Bar`** command is used to show or hide the **Status Bar** (Figure 24), which is located at the bottom of the GUI. If the **Status Bar** is shown, it is indicated by a highlighted check mark left hand to the command.

The **Status Bar** contains the type of currently loaded method, the active feature button of the **Toolbar,** the coordinates of the mouse pointer and the Status Information concerning the state of the instrument.

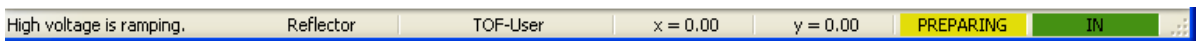


Figure 24 Example of a Status Bar

3.4.4 Tools Menu

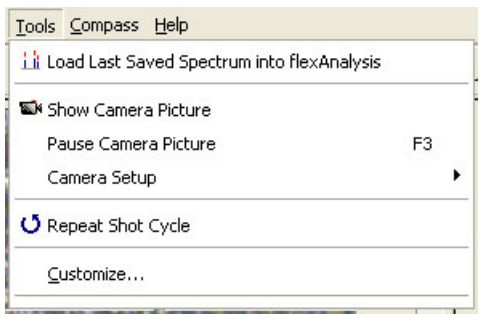


Figure 25 Contents of the Tools Menu

The **Tools** Menu (Figure 25) is composed of various commands for different features, e.g., to re-align the video camera, choose a post processing application, etc.

3.4.4.1 Overview

Table 4 Feature buttons of the Tools menu with related functions

Feature button	Tool tip text	Tools Menu commands	Shortcut	Description
	Load Last Spectrum into **flexAnalysis**	Load last saved spectrum into post-processing application		Transfers the last saved spectrum directly into **flexAnalysis**.
	Camera	Show Camera Picture		Toggles spot symbol and true video
		Pause Camera Picture	F3	Corresponds to Pause on a video recorder.
	Camera teaching	Teach Camera Control Features		Opens a dialog for camera adjustments.
	Repeat shot cycle	Repeat shot cycle		Repeats the last shot sequence until termination.
		Customize Online Processing		Parameterizes the peak finders.
		Customize		Customizes the Toolbar.

3.4.4.2 Load Last Spectrum Into flexAnalysis

The **`Load Last Spectrum Into flexAnalysis`** command and the corresponding icon of the **Toolbar** are used to transfer the last saved spectrum directly into this post-processing software.

3.4.4.3 Show Camera Picture

The **`Show Camera Picture`** command and the corresponding icon on the **Toolbar** allow toggling between spot symbol and true video of the **Target Manipulation Segment**.

3.4.4.4 Pause Camera Picture

The **`Pause Camera Picture Command and`** the short cut (**`F3`**) are used to pause the video signal. In **flexControl** this is shown under the video (Figure 26). During Webex sessions it is very useful to pause the video signal to have faster data transfer.

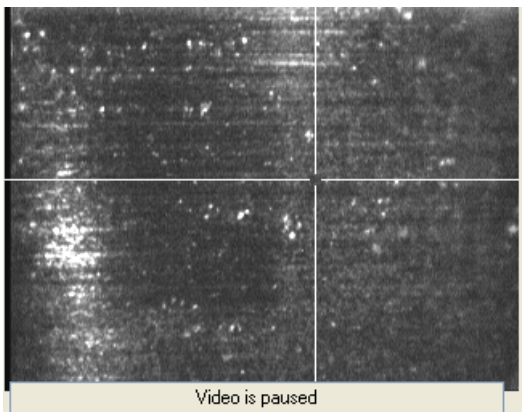


Figure 26 Paused video picture

3.4.4.5 Teach Camera Control Feature

The **`Show Teach Camera Control Feature`** command and the corresponding icon on the **Toolbar** are used to open the dialog shown in Figure 27 for matching target size with the camera position.

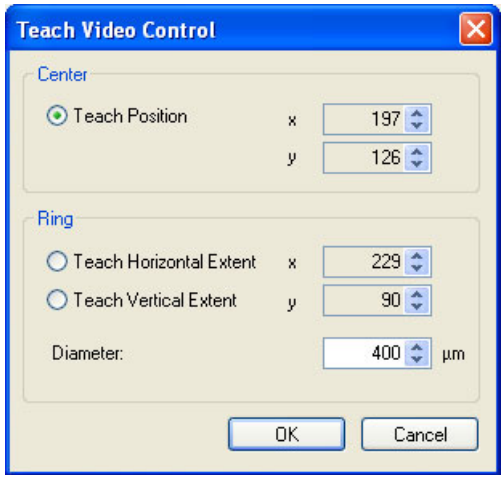


Figure 27 Parameters to match target size and camera position

The feature **Teach Video Control** (Figure 27) is an alignment tool in order to match the screen coordinates and respective motor positions. The 1000 µm radius refers to the sample spot of the standard SCOUT MTP target plate.

To perform video teaching the coordinates of the crosshair have to be re-determined and the blue ellipse-shaped calibration marker has to match the rim of the sample spot. Since the oblique incident laser beam causes an ellipse-shaped projection on the target plate, two components (x and y) of the border must be readjusted.

Matching target size and camera position:

1. Press the button “Show Camera Picture” to display the true video picture.
2. Apply **`Teach Video Control`**. This function superimposes the related window with the components to be re-calibrated and the blue crosshair with the related ellipse-shaped border.
3. Select the radio button of the component, which shall be re-calibrated first. Default is **Teach Position** of the ‘Center’ group box.
4. For example, altering the position of the crosshair is performed with the mouse pointer either by clicking on the target or using the respective spin buttons. The displayed values of the grayed field follow the entries; however there is no direct access to the field.
5. If necessary proceed with the components of the ‘Ring’ group box by selecting the corresponding radio button (x and/or y).
6. Store with “OK”.

3.4.4.6 Repeat Shot Cycle

The **`Repeat Shot Cycle`** command, or the corresponding icon of the **Toolbar** are used to repeat the last shot sequence until the command or the button are applied a second time.

3.4.4.7 Customize

The **`Customize`** command opens the Customize dialog (Figure 28). It is used to vary the contents of the Menu bar. Additionally the user can remove or add parts of the toolbar.

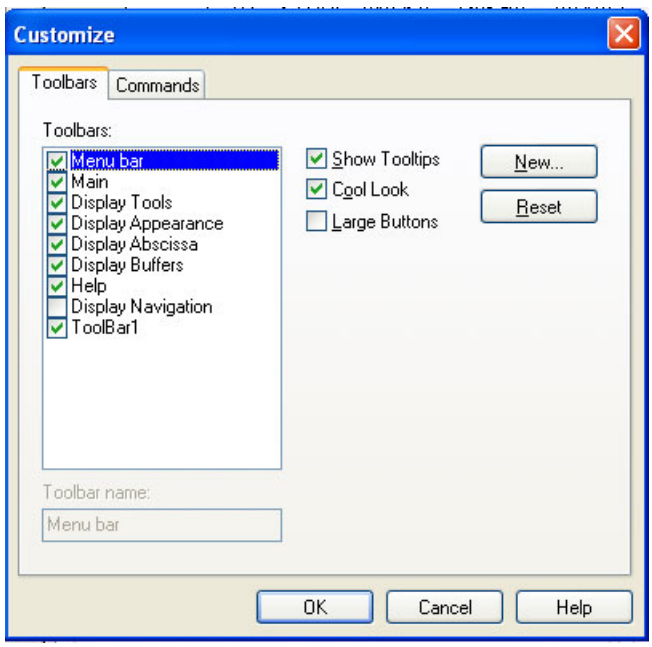


Figure 28 Customize Toolbars dialog

3.4.5 Compass Menu

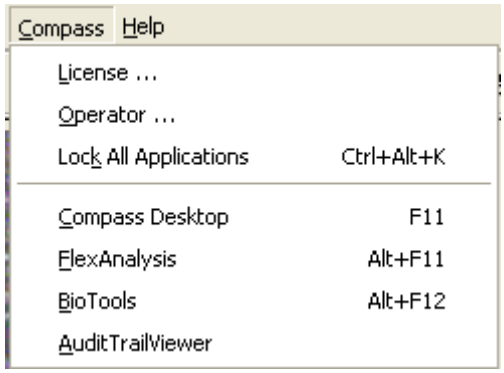


Figure 29 Content of the Compass menu

3.4.5.1 Overview

Table 5 Features of the Compass Menu

Feature button	View Menu commands	Shortcut	Description
	License		Enter the License number here.
	Operator		Changes the operator.
	Lock all Applications	Ctrl + Alt + K	Locks all programs in case the Compass Security Pack is installed.
	Compass Desktop	F11	Opens the Compass Desktop.
	FlexAnalysis	Ctrl + F11	Opens flexControl.
	BioTools	Alt + F12	Opens BioTools.
	Audit Trail Viewer		Opens the Audit Train Viewer in case the Compass Security Pack is installed.

3.4.5.2 License

The **`License`** command is used to open the Bruker Daltonics License Manager dialog box (Figure 30) for viewing or adding/removing licenses for Bruker products. The

License Manager looks the same in nearly all programs. It is not necessary to enter a license for a special Bruker program in the respective program; it can be entered via the License Manager of other Bruker programs.

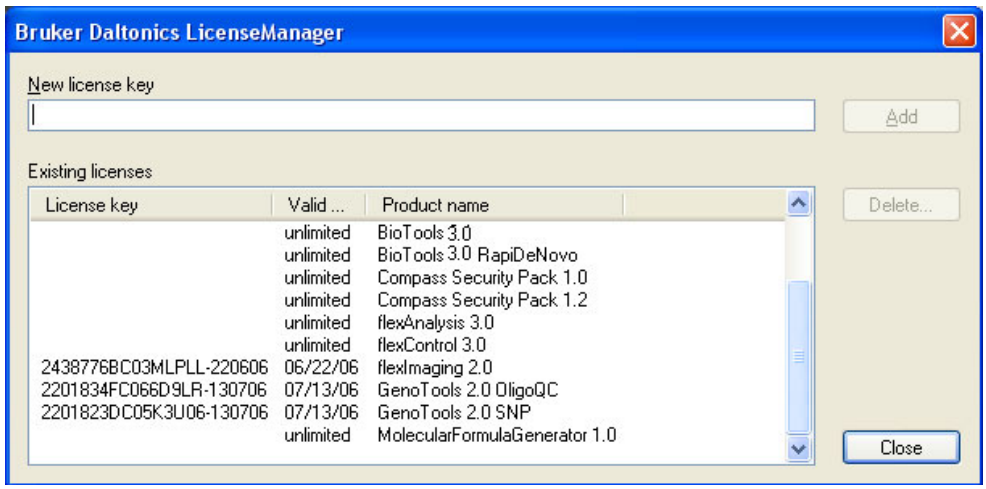


Figure 30 The License Manager

3.4.5.3 Operator

The **`Operator`** command opens the dialog shown in Figure 31 and is used to log on as new operator. The new operator name appears in the Status bar at the bottom of the GUI. In case the Bruker Daltonics UserManagement is installed additionally a password is requested.
If a user logs on with the **`Operator`** command the user automatically changes for all other Bruker programs that are currently open and run with the UserManagement. In **flexControl** the **Select Method** dialog comes up.

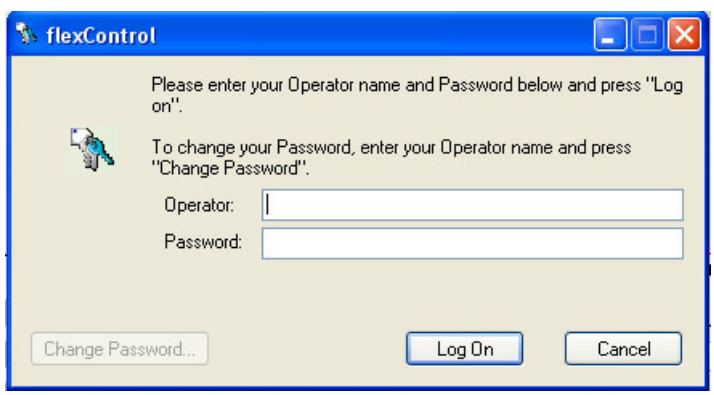

flexControl
Please enter your Operator name and Password below and press "Log on".
To change your Password, enter your Operator name and press "Change Password".
Operator:
Password:
Change Password...
Log On
Cancel

Figure 31 Log On operator dialog

If the Bruker UserManagement is not installed the dialog shown in Figure 32 appears.

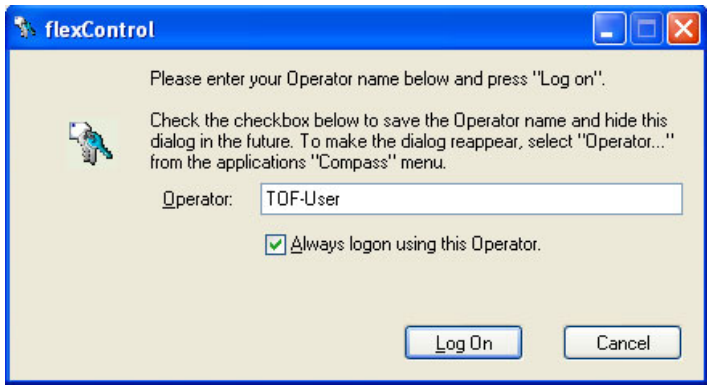

flexControl
Please enter your Operator name below and press "Log on".
Check the checkbox below to save the Operator name and hide this dialog in the future. To make the dialog reappear, select "Operator..." from the applications "Compass" menu.
Operator:
TOF-User
Always logon using this Operator.
Log On
Cancel

Figure 32 Conventional Log On operator dialog

3.4.5.4 Lock All Applications

The **`Lock all Applications`** command and the short cut (**`Ctrl + Alt + K`**) lock all the applications that are currently open depending on the lock-time that is defined in the UserManagement. Additionally the Unlock dialog opens. After a timeout, that is adjustable via the UserManagement, the software is locked automatically.

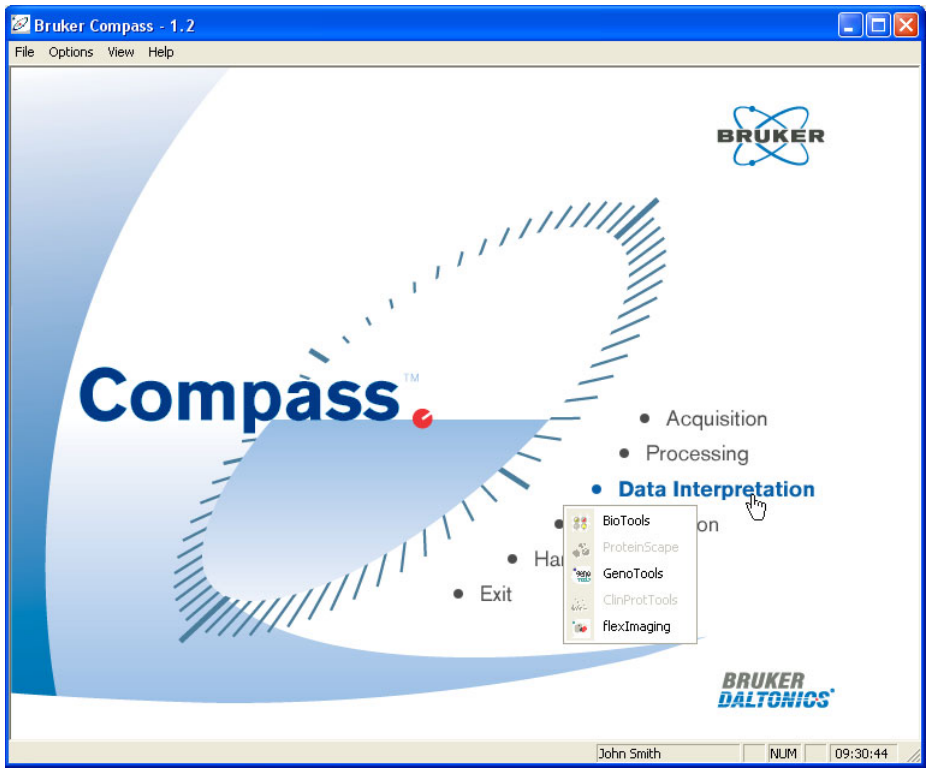
If one program is locked (manually or via timeout), all programs that run with the Usermanagement are locked, since they all use the same UserManagement server. In this case it is also necessary to un-lock only one program. A locked program can only be un-locked from the user who locked it, or from the Usermanagement administrator.

flexControl
This application was locked by John Smith. It can be unlocked by the previous operator or someone with the special right to unlock applications.
Please enter your Operator name and Password below and press "Log on".
Operator:
Password:
Change Password...
Log On
Cancel

Figure 33 Unlock applications dialog

3.4.5.5 Compass Desktop

The **`Compass Desktop`** command opens the so called Bruker Compass. This GUI offers access to the installed Bruker programs distributed in Acquisition, Processing, Data Interpretation, and the belonging manuals.



3.4.5.6 FlexAnalysis

The **`FlexAnalysis`** command is used to open **flexAnalysis** or bring it to front if it is already open. Data transfer is not achieved with this command, i.e. no spectrum is sent to **flexAnalysis**. To send a spectrum use the button **`Load last saved spectrum into flexAnalysis`** (section 3.4.4.2).

3.4.5.7 BioTools

The **`BioTools`** command is used to open BioTools or bring it to front if it is already open. Data transfer cannot be achieved with this command, i.e. no spectrum is sent to BioTools since BioTools works with peaklists but **flexControl** does not create them.

3.4.5.8 Audit Trail Viewer

The **`Audit Trail Viewer`** command opens the Bruker Daltonics AuditTrailViewer dialog. It is only available if the Bruker Usermanagement is installed.

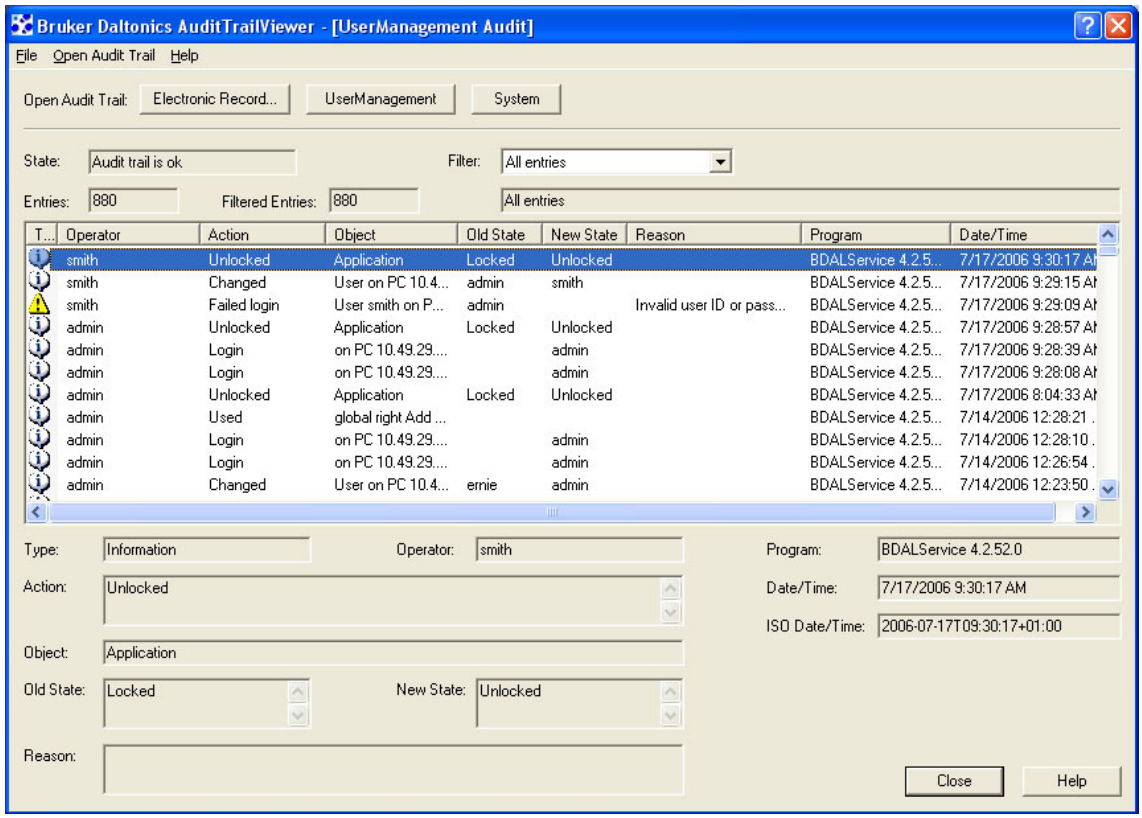

Bruker Daltonics AuditTrailViewer - [UserManagement Audit]
File Open Audit Trail Help
Open Audit Trail: Electronic Record... UserManagement System
State: Audit trail is ok
Filter: All entries
Entries: 880
Filtered Entries: 880
All entries
T... Operator Action Object Old State New State Reason Program Date/Time
smith Unlocked Application Locked Unlocked BDALService 4.2.5... 7/17/2006 9:30:17 AM
smith Changed User on PC 10.4... admin smith BDALService 4.2.5... 7/17/2006 9:29:15 AM
smith Failed login User smith on P... admin Invalid user ID or pass... BDALService 4.2.5... 7/17/2006 9:29:09 AM
admin Unlocked Application Locked Unlocked BDALService 4.2.5... 7/17/2006 9:28:57 AM
admin Login on PC 10.49.29.... admin BDALService 4.2.5... 7/17/2006 9:28:39 AM
admin Login on PC 10.49.29.... admin BDALService 4.2.5... 7/17/2006 9:28:08 AM
admin Unlocked Application Locked Unlocked BDALService 4.2.5... 7/17/2006 8:04:33 AM
admin Used global right Add ... BDALService 4.2.5... 7/14/2006 12:28:21 .
admin Login on PC 10.49.29.... admin BDALService 4.2.5... 7/14/2006 12:28:10 .
admin Login on PC 10.49.29.... admin BDALService 4.2.5... 7/14/2006 12:26:54 .
admin Changed User on PC 10.4... ernie admin BDALService 4.2.5... 7/14/2006 12:23:50 .
Type: Information
Operator: smith
Program: BDALService 4.2.52.0
Action: Unlocked
Date/Time: 7/17/2006 9:30:17 AM
ISO Date/Time: 2006-07-17T09:30:17+01:00
Object: Application
Old State: Locked
New State: Unlocked
Reason:
Close
Help

Figure 34 The Audit Trail Viewer

3.4.6 Help Menu

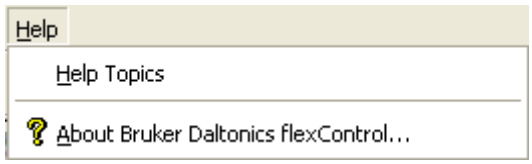


Figure 35 Contents of the Help menu

The **Help** Menu (Figure 35) is composed of commands referring to copyright and license information, and to electronic support.

3.4.6.1 Help Topics

The **`Help Topics`** command opens the online-help system and displays the help system's contents page.

3.4.6.2 About Bruker Daltonics flexControl

This command opens a window (Figure 36) containing the software version, copyright information, the used mass spectrometer, and the support address.

Figure 36 The About box

3.4.7 Main Instrument Control

This part of the **flexControl** GUI comprises features of the Video, data acquisition and method information as well as the Sample Carrier.

3.4.7.1 Video Optics and Acquisition Control

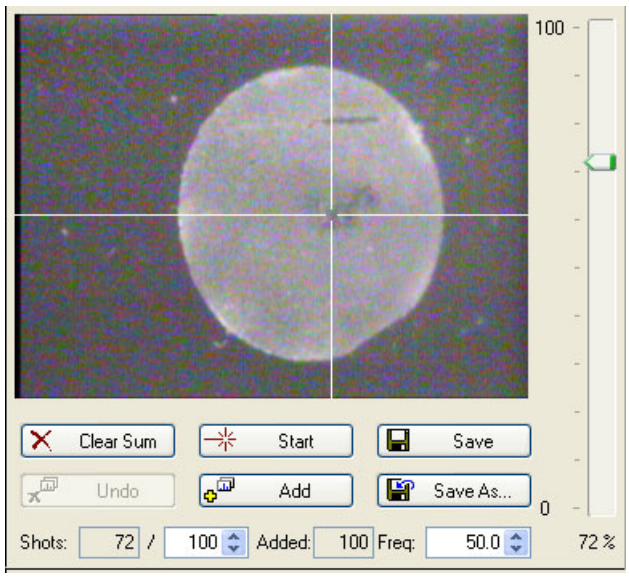
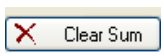


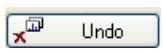
Figure 37 Video optics and acquisition control

The slider on the right of the video picture is used to adjust the laser intensity. This can be done with mouse clicks or with the mouse wheel since the focus is on the laser slider after a click in the video picture.

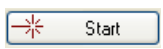
Beneath the video six buttons are found to handle data acquisition and collection, and a field displaying the laser power.



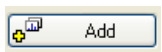
A click with the left mouse button will erase the contents of the linear and reflector sum buffers.



Deletes the last added shot sequence from the sum buffer.

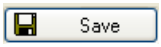


Starts a manual acquisition. During a run it toggles to a Stop-button, which might be used to terminate the shot sequence immediately.

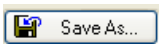


Transfers the current contents of the linear or reflection detector to the corresponding sum buffer. The data will be added to data previously acquired. This should be done only with good spectra in

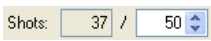
order to maximize the quality of the sum spectrum.



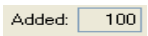
Saves the current spectrum.



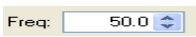
Saves the current spectrum with a new name.



Information on how many shots are currently acquired and how many are required.



How many shots have been added to the sum buffer.



Shows the currently used percentage of the maximum laser power.

3.4.7.2 MTP Control and Method Selection

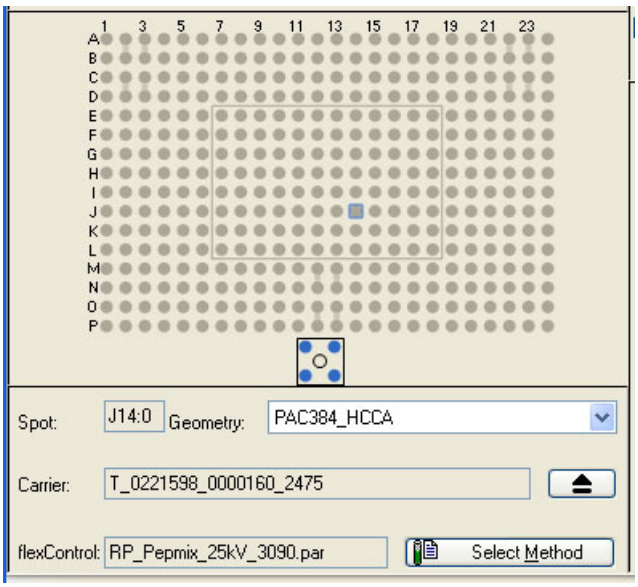
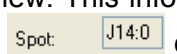
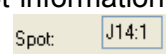


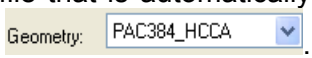
Figure 38 Target control features

Depending on the target that is currently used in the instrument the respective geometry is presented. If it is a target with chips, as shown in Figure 38, the different chips that are available are visualized beneath the target ( ) and can be chosen with a click on a circle. The currently active chip (Figure 38: chip 0) is shown in blue. In the example shown in Figure 39 “chip 1”, i.e. the calibration spots are selected.

The currently active *sample spot* is marked with a blue (system color dependent) square in the plate view. This information is additionally shown in the Spot information field. In the example 

denotes J14, chip 0, i.e. a sample spot. 

identifies J14, chip 1, i.e. the calibration spot.

The geometry file that is automatically loaded for transponder targets is shown in the Geometry field 

. If a target geometry is detected during the load process, the Geometry filed becomes read-only. The only exception are PAC targets, due to the fact, that different (disposable) targets can be used on the same PAC frame (adapter).

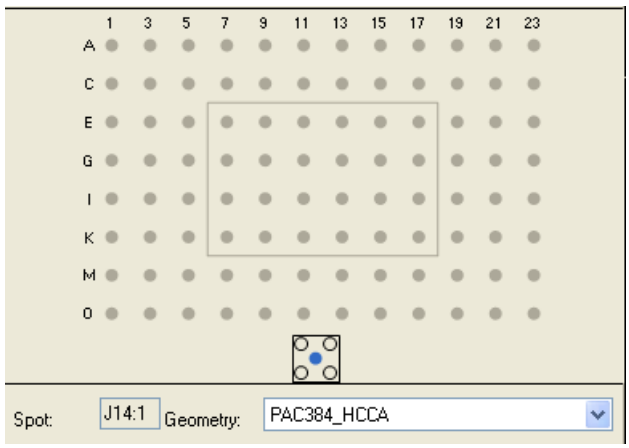


Figure 39 PAC target with selected calibration chip

During an AutoXecute run when **flexControl** acquires and saves data, the appearance of the Plate View gets colored. This depends of the measurement status of the spots. Two main states are available and will be saved in the run:

- light green: MS measurement finished
- dark green: MS/MS measurement finished

Additionally four other states can be shown before and after the run. These states can only be seen on the plate view, they are not permanent!

- white: prepared MS and MS/MS positions
- mauve: prepared calibration spots
- red: unsuccessful acquisition
- orange: flatline spectrum saved

If a spot is green colored (light or dark) this denotes that a spectrum has been acquired and saved either automatically or manually with adding information to a run (see section 3.4.1.2, **Run** and **Add to Run**). In both cases the *measurement status* has also been saved in the AutoXecute run. For this reason it is possible to send spectra to **flexAnalysis** with right-mouse-click on the colored sample spot.

3.4.8 Mass Spectrum Window

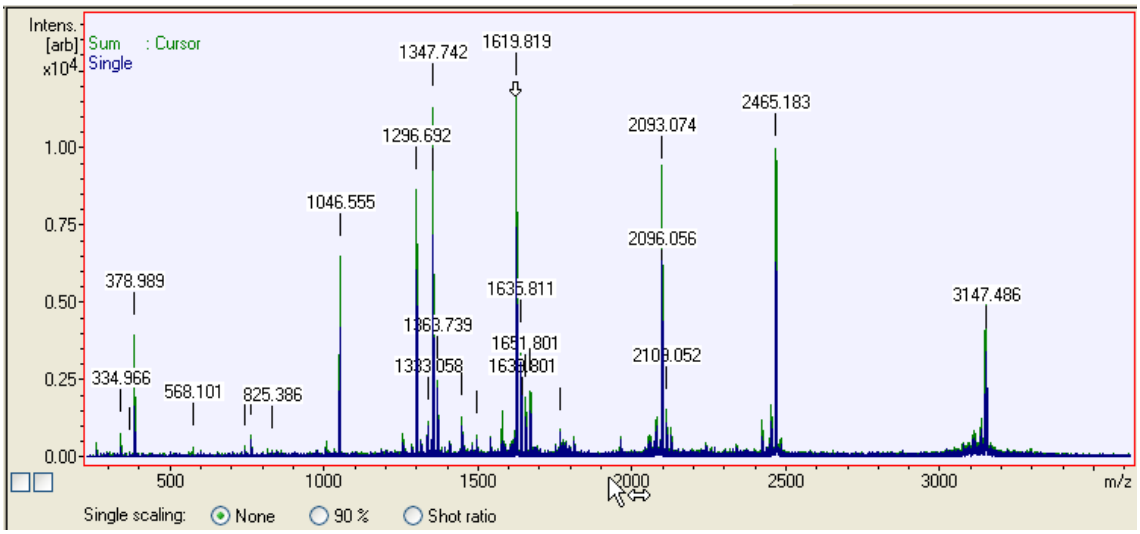


Figure 40 Example of a spectrum

The **Mass Spectrum Window** (Figure 40) is located top right on the GUI. It displays the currently acquired spectrum.

flexControl automatically normalizes the peak intensity scale. The x-axis may be scaled with one of the three available units (covered in section 3.4.2.11). Pre-selection of the scale can be performed on the **Menu Bar** by clicking the command **`Display|Abscissa Units`** (section 3.4.2.11).

The **Mass Spectrum Window** can be divided in up to three windows (Figure 21). This may be useful to display simultaneously a full spectrum and two different zoomed areas.

The two check boxes down left are used for auto-scaling both the ordinate and the abscissa.

The function of the three scaling radio button below the x-axis is covered in section 3.4.2.12.

To enhance the viewing of data the color of one or more objects in the **Mass Spectrum Window** may be changed. For this refer to section 3.4.8.2.

3.4.8.1 Scaling Pointers

To shift the currently displayed spectrum either in x- or in y-direction **flexControl** provides two additional pointers:

- **Horizontal Scaling pointer:**

  This pointer appears below the abscissa of the Mass Spectrum Window.

  It is used to shift the spectrum and the scale horizontally.

- **Vertical Scaling pointer:**

  This pointer appears below the ordinate of the Mass Spectrum Window. It is used to shift the spectrum and the scale vertically.

3.4.8.2 Spectrum Manipulation

A right mouse click within the **Mass Spectrum Window** opens a context menu shown in Figure 41. These features are used to customize the peak finders and the Real Time Display.

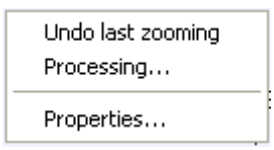


Figure 41 Content of the right-click pop-up menu

- **`Undo last zooming:`**

  Please refer to section 3.4.2.3.

- **`Processing:`**

  This command opens the Processing Method Editor. Please refer to section 3.4.9.5.

- **Properties:**

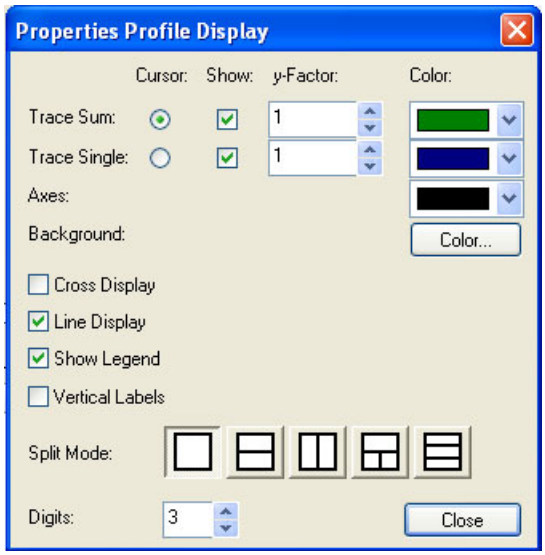


Figure 42 Features of the Properties dialog

The dialog **Properties** (Figure 42) may be used to configure in advance the **Mass Spectrum Window(s)** (section 3.4.2.13) and to display spectra in different ways.

- **Trace Single/Sum**: On selecting the respective checkboxes spectra of the single and/or sum buffer are shown in the **Spectrum Window**. Clearing the boxes will fade out the spectra. The scale of the y-axis and the trace colors are to be adjusted in this dialog.
- **Axes**: A color can be specified for both the x- and y-axis.
- The button “Color” opens the dialog shown in Figure 43, which is used to assign colors to th

  Colors can be selected either from a set of basic colors or from a set of custom colors which the operator can define according to his own requirements.

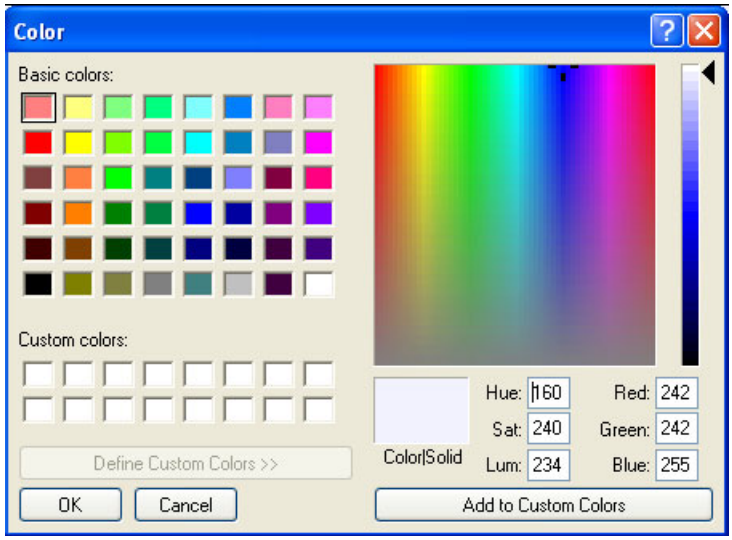


Figure 43 The palette

- "Cross display": When selected, the spectrum is shown as a series of crosses, each cross representing a measuring point.
- "Line display": When selected, all measuring points of the spectrum are connected and shown as a line.
- "Show legend: If selected, an information line is added on top of the RTD.
- "Vertical labels": When selected, labels are displayed vertically.
- **Split Mode**: Pre-selection of how much Mass Spectrum Windows are displayed.
- **Digits**: Pre-selects the decimals of the peak labels.

3.4.9 System Configuration Segment

Depending on the currently loaded method **flexControl** provides respective pages or specific features in pages located in the **System Configuration Segment** (Figure 3):

- ***AutoXecute*** (section 3.4.9.1),
- ***Sample Carrier*** (section 3.4.9.2),
- ***Spectrometer*** (section 3.4.9.3),
- ***Detection*** (section 3.4.9.4),
- ***Processing*** (section 3.4.9.5),
- ***LIFT*** (section 3.4.9.10), shown in case a LIFT method is loaded
- ***FAST*** (section 3.4.9.11), shown in case a FAST method is loaded
- ***Setup*** (section 3.4.9.6),
- ***Calibration*** (section 3.4.9.8),
- ***Status*** (section 3.4.9.12)

The ***LIFT*** page (section 3.4.9.10) and the LIFT ***Calibration*** page (3.4.9.9) appear if a **flexControl** LIFT-method is loaded.

The ***FAST*** page (section 3.4.9.11) is only available, if previously a FAST-method has been loaded.

Note: New entries in the edit fields on all tabs only take effect after having hit the <**Enter**> key on the keyboard. The color of the new entry changes from blue to black.

3.4.9.1 AutoXecute

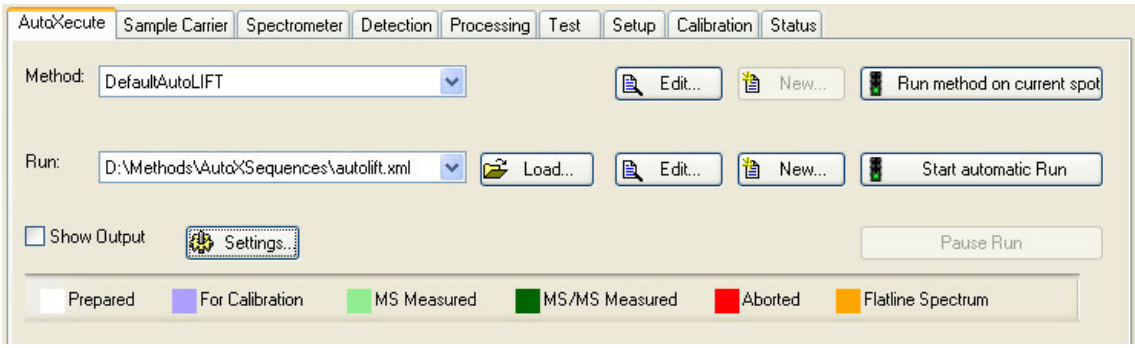


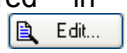
Figure 44 Features of the *AutoXecute* page

The ***AutoXecute*** page (Figure 44) is used to create, edit and load AutoXecute methods and runs (sequences), to start, pause and stop AutoXecute runs and to configure some general settings concerning AutoXecute.

AutoXecute allows setup and running of automatic runs with data acquisition in **flexControl**, calibration, processing and annotation in **flexAnalysis**, and database search in **BioTools** or **ProteinScape**.

3.4.9.1.1 AutoXecute Methods

For automatic data acquisitions in **AutoXecute** Bruker provides pre-installed write-protected methods. The method names start with <Default>. All pre-installed methods are deleted when a new version of the program is installed. Therefore it is strongly recommended to use the default methods as templates to create own methods with user-defined names. These methods can be modified at any time and are not touched during de-installation. The tool for method development is the **AutoXecute Method Editor**.

The Method selection box on the ***AutoXecute*** page (Figure 44) offers all available AutoXecute methods that are stored in the correct directory (typically D:\Method\AutoXmethods). The button 

opens the **AutoXecute Method Editor** with the selected method. It consists of seven pages providing a variety of functions to customize automatic data acquisition. These functions are described in the following. To create a new AutoXecute method, select an existing one and save it with a new name.

General: General settings, as method selection and comments

Laser: Adjustment of the laser (Fuzzy Control)

Evaluation: Customize the spectra evaluation

Accumulation Customize accumulation Fuzzy Control

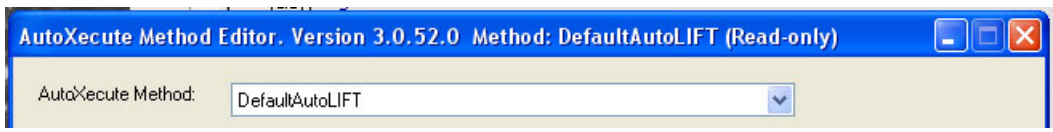
Movement: Specify the movement on the sample

Processing: Specify post processing settings

MS/MS: Setting parameters for an automatic MS/MS measurement

AutoXecute Method:

The drop down list on the top of the AutoXecute method editor shows the currently loaded **AutoX** method. The selection remains unchanged when switching between the tabs.



If another method should be loaded it can be selected from the list offered here. If changes in the current method have not been saved a warning message appears and asks for saving.

***General* tab**

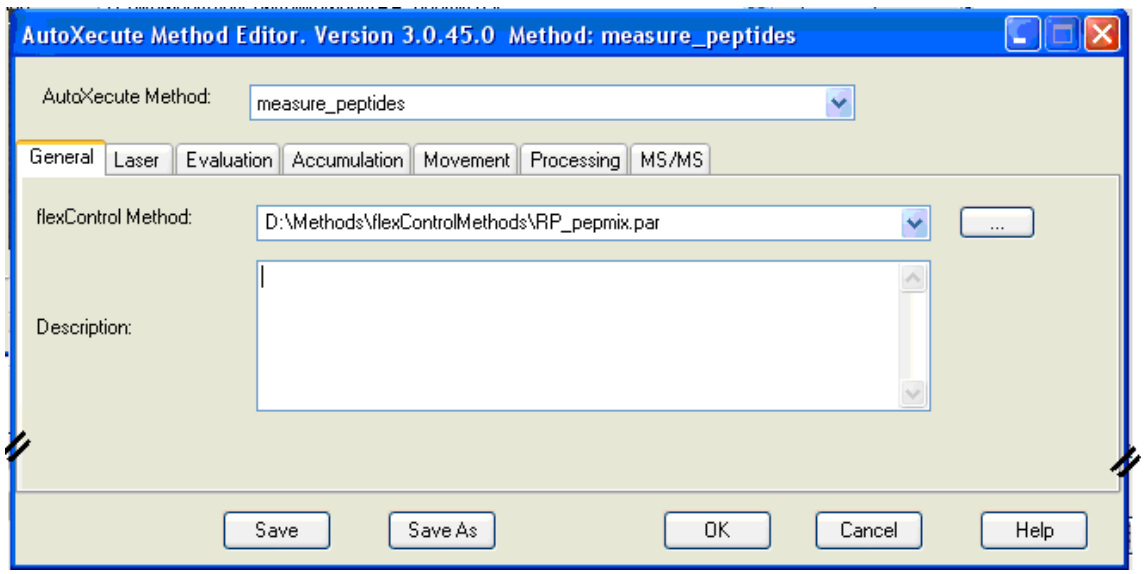


Figure 45 Content of the *General* page

The **General** page (Figure 45) is used to perform general settings.

- **flexControl** Method:

  Depending on the chosen flexControl method the AutoXecute method becomes a method for MS or MSMS measurement. Some parameters are then disabled because they are not relevant for the selected usecase. If a formally chosen **flexControl** method is no longer available on the hard drive its name appears red colored and a warning message appears when the AutoX method is saved.

  For the MS usecase it is possible to choose “None”, i.e. no **flexControl** method. In those cases the method currently loaded in **flexControl** (section 3.4.7.2) is used for measurement. If it is no MS method (but LIFT or FAST) a warning appears in the AutoX output (0) and the measurement will not start.

- **Description**:

  This field can be used for comments.

Laser tab

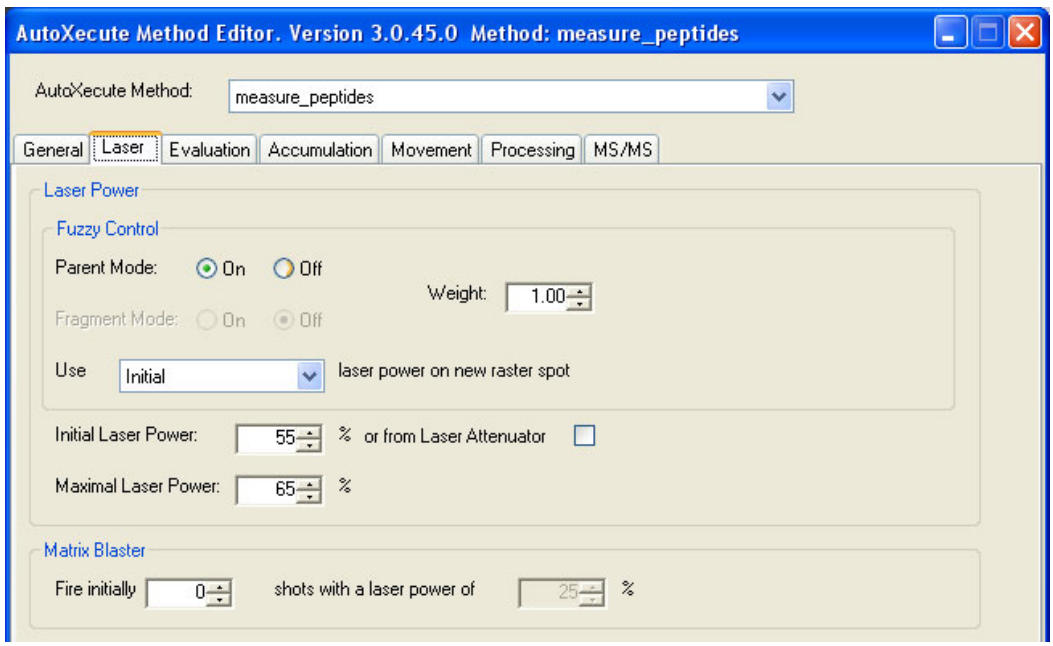


Figure 46 Content of the *Laser* page

The ***Laser*** page (Figure 46) contains features to configure the laser power using the advantage of **Fuzzy Control**. All the entries have a global character and are therefore valid during the entire acquisition.

- Group box ‘Laser Power’:

  The module **Fuzzy Control** works like an automatic regulation circuit. It measures and processes two control arguments, such as peak resolution and peak intensity (S/N). As a result it delivers two output values, in this tab to optimize the laser intensity, and accumulate spectra to the sum buffer, which are considered good enough.

  In case of a MS/MS method, the **Fuzzy Control** can separately be switched on and off for parent and fragment measurement. The required parameter settings are accessible if a **flexControl** LIFT method has been selected.

  Data recording of fragmented LIFT spectra can be controlled by the Fuzzy module analogue to the handling of MS data. Fuzzy logic for fragments handles fragmented spectra in the same manner as peptide fingerprint spectra.

  In case of “Fragment Mode Off” the entered number of laser shots (Edit field **Shots**) with the boosted laser power adjusted on the ***Lift*** page of the **flexControl** GUI (Figure 93) is fired.

  Weight is a parameter of the **Fuzzy Control** engine to scale the laser power. It is a configurable term of a couple of factors that are associated with the step width of the laser power changing gradually from the “Initial Laser Power” to the “Maximal Laser Power”. Variations are possible in minimum steps of **0.1** in the range between **0.5** and **2.0**. The values in the pre-installed default methods are the recommended ones for the respective usecases.

- ‘*Use….*’:

  This feature is used to specify the laser power that is used on a new *raster* spot (measuring position on a sample spot) if the current raster position has to be left (due to restrictions set in the AutoX method) but the required sum of spectra from this sample spot has not been reached yet (concerning the definition of sample and raster spots, refer to page3-53). The four possible choices are:

  1. Set the laser power to the **initial value** for this raster spot. This is either the value defined below (“Initial Laser Power”), or the one from the Laser slider in **flexControl**, in case the checkbox “from Laser Attenuator” is activated.
  2. Set the laser power to the **first successful** acquisition that was done on the previous raster spot. If there was no successful acquisition on the previous raster spot, the laser power will be optimized in a way as if the previous raster spot has not been left. For example, if you do not know in which range the optimum laser power is, then type is a low laser power and choose first successful. Then the laser power will be increased over several raster spots to the optimum.

3. Set the laser power to the **last successful** acquisition that was done on the previous raster spot.
4. As soon as a shot package has been acquired **successfully**, this laser power is **locked** for all following shots until the number of required shots on the sample spot is reached; no evaluation of the shot packages takes place. A raster spot is left if the number of allowed shots per raster spot is reached.

The **Initial Laser Power** field determines the laser power (in %) that is used to start the acquisition on a *sample* spot.

If the checkbox ”from Laser Attenuator” is selected, an external value for laser power, taken from the **flexControl** GUI, will be activated. The value for **Initial Laser Power** is then disabled. If the value imported from **flexControl** is more intensive than the previously set value for **Maximal Laser Power**, the latter value will be used. So be careful with this setting and remember that the last used laser power on one *sample* spot would be used as starting laser power on the next *sample* spot!

- Group box ‘Matrix Blaster’:

  If it is necessary to get rid of the first substance layer of a new sample spot, which shall not be measured, the function of **Matrix Blaster** may be used by firing a number of laser shots with a specific intensity. Data obtained from here are ignored. Default is **0** shots and **0** laser power that means this function is disabled.

Evaluation tab

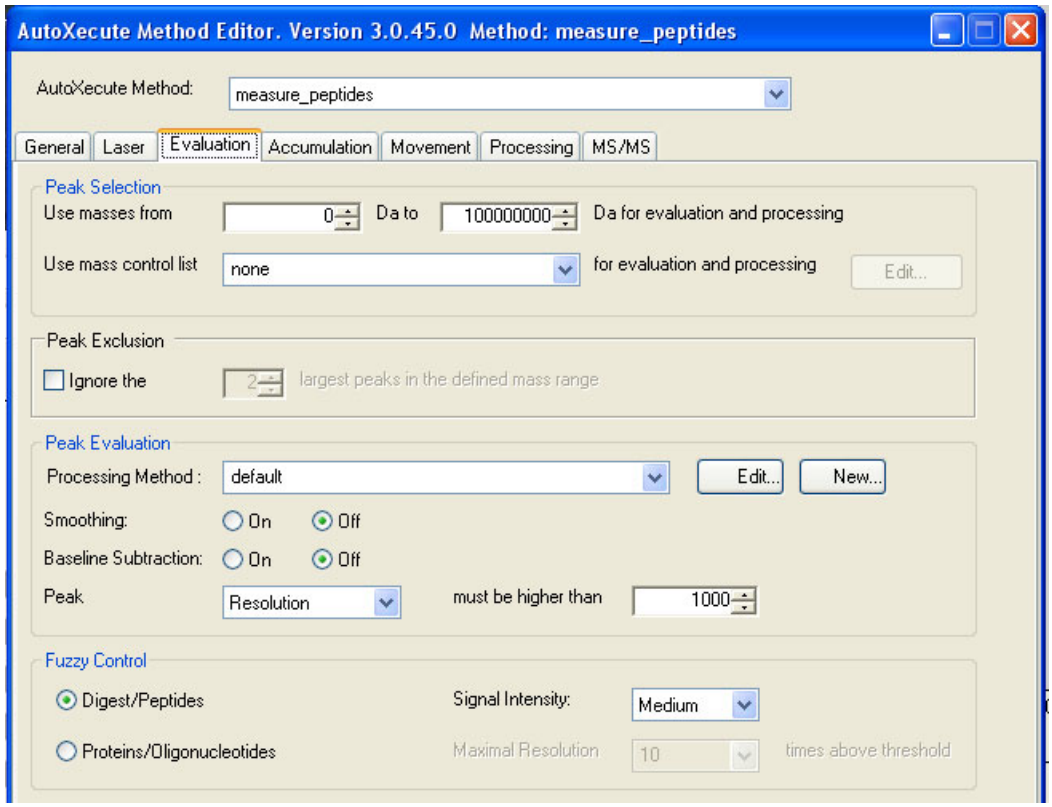


Figure 47 Content of the *Evaluation* page

The tab ***Evaluation*** (Figure 47) is used to configure the second output of the fuzzy engine for peak evaluation and processing.

- Group box ‘Peak selection’:

  The row **Use masses from x Da y Da to for evaluation and processing** is used to define a mass range, which shall be evaluated during measurement. This has no influence on the acquisition mass range defined in the **flexControl** method (section 3.4.9.4).

  If some specific peaks should not be evaluated during an AutoXecute run, you have to create a mass control list, where these masses are defined as background (Figure 48). Since Centroid should be used as evaluation peak detection algorithm (every isotope is annotated) the fix tolerance of ±2000 ppm is applied, so it is not necessary to enter all isotope masses in the list.

The same masses and as well the fixed tolerance of ±2000 ppm is used, if AutoXecute filters the precursor masses. This means, that there will be no MSMS measurement of masses, which are defined as background masses in this list.

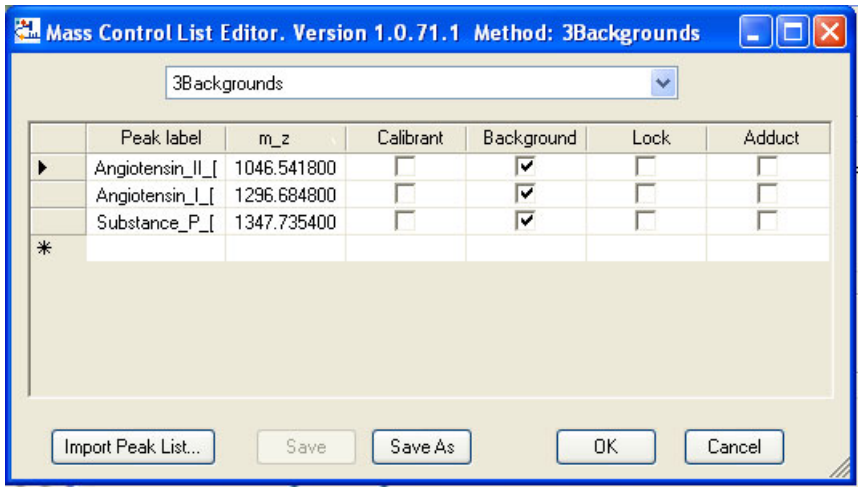


Figure 48 Mass Control List Editor for AutoXecute

- The column *Peak label* contains the names of the substances
- The column *m/z* contains the theoretical values of the respective peaks.
- The columns *Calibrant*, *Background*, *Lock* and *Adduct* contain checkboxes to assign a mass to a specific purpose. For AutoXecute the Background column is important. An access to the content of the column **Lock** will be implemented in a future version of **flexControl**.

- Group box 'Peak Exclusion':

  If the checkbox is activated the entered number of *highest* peaks (from the defined mass range) is ignored during the evaluation. Even here the fix tolerance of ±2000 ppm is used. If a background list is additionally selected, first the backgrounds are excluded, afterwards the **x largest peaks**. Detailed information are given in the AutoX Output during the run.

- Group box 'Peak Evaluation':

  The peakfinder and related parameters used for *evaluation* during an AutoXecute run are offered via the **Processing Methods** that are already known from **flexControl** and **flexAnalysis.** It is recommended to use Centroid as peak detection algorithm. The used Signal/Noise Threshold is related to the highest peak. This one must exceed this level otherwise the spectrum will be

ignored. During the automatic run the **Processing Method** chosen in the AutoXecute method is transferred to **flexControl** (Processing tab).

Smoothing may be applied to filter out spikes from a spectrum. This process is performed before the fuzzy module judges the spectrum.

If the parameter **Baseline Subtraction** is applied, the baseline is subtracted before the peak judgment operation is performed.

Note: *Smoothing* and *Baseline Subtraction* are only applied for judging in AutoXecute. If a so modified spectrum is accepted, the original (unmodified) spectrum is added to the sum buffer.

Entries in the last row concern the **Peak Resolution** or alternatively the **Half Width** of the most prominent peak of the current spectrum. It is recommended to use Half Width for LIFT measurements, since the Half Width of parent and fragments is comparable, in contrast to the respective Resolutions.

Detailed information on the peak that is currently evaluated is given in the AutoXecute output

- Group box 'Fuzzy Control':

  AutoXecute provides specific fuzzy engines for two substance classes. One suits best for measuring peptides the other one is right for measuring proteins.

  The radio button "Digest/Peptides" is chosen for peptides in case of smaller molecules and high resolution. The combo box **Signal Intensity** contains criteria to specify a kind of threshold (Intensity per shot) peaks must exceed to be accepted. Four thresholds can be chosen: High (20), Medium (10), Low (5) and Very Low (2). From the AutoX Output the Intensity per shot can be seen for the evaluated peak.

  The fuzzy engine chosen with the radio button "Proteins/Oligonucleotides" performs best with greater molecules and poor resolution. To avoid identifying noise as a peak a maximal resolution can be specified in the combo box **maximal Resolution**. The chosen resolution in the row **Peak** above is the reference.

Accumulation tab

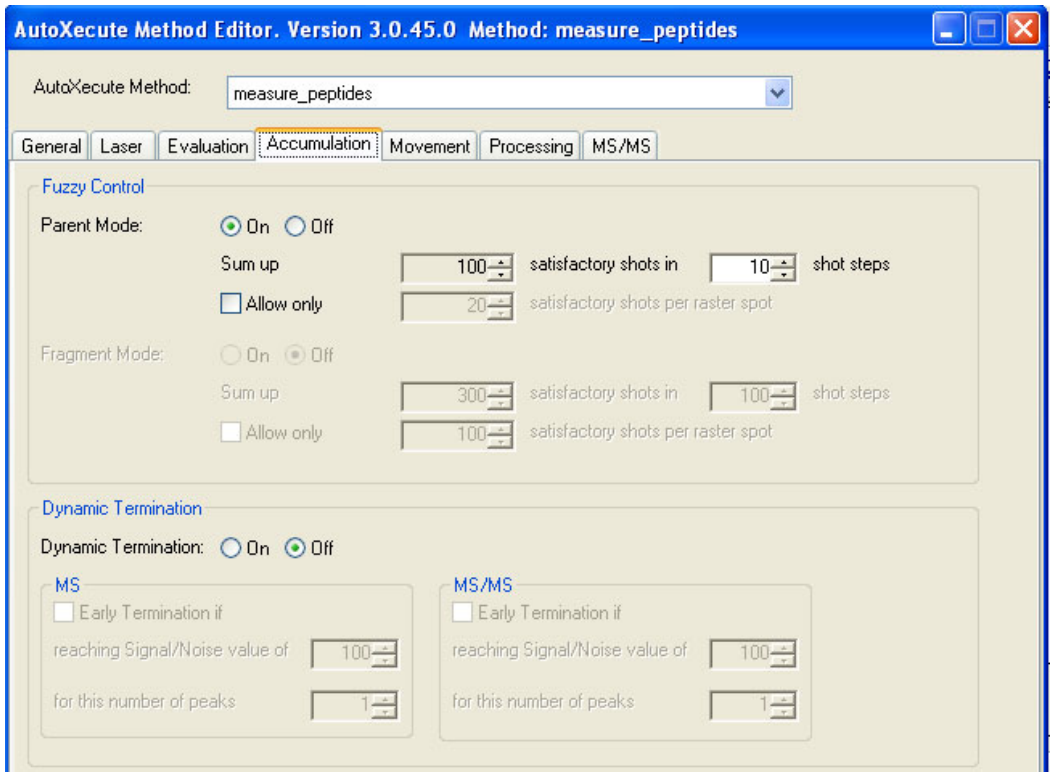


Figure 49 Content of the *Accumulation* page

The **Accumulation** page (Figure 49) is used to determine by means of the fuzzy engine, whether spectra are added to the sum buffer.

When **Fuzzy Control** is switched off, any recorded spectrum is added, regardless of its quality. This is the same for MS or parents and fragments in the MS/MS mode.

If the **Allow only** checkbox is activated, the raster spot is changed after the given number of satisfactory shots has been *added*.

Note: The mass range for the evaluation of the fragment spectra starts at 200Da and ends at “parent mass – 200Da” (default value). It can be changed in the AutoXecute Settings dialog (section 3.4.9.1.6).

The **Dynamic Termination** (Figure 50) feature offers the possibility to end the acquisition on a sample (if the spectrum is already good enough) before the required number of shots is reached: The already added shot packages in the sum buffer are evaluated after each new addition. If the defined Signal/Noise threshold for the given number of peaks is reached the acquisition on this sample spot is complete. This feature works *together* with the common evaluation feature. So, a sample spot will be

left as soon as one of the thresholds (Evaluation tab or “Early Termination”) is reached. The feature can separately be used in the MS mode as well as during MS/MS measurement.

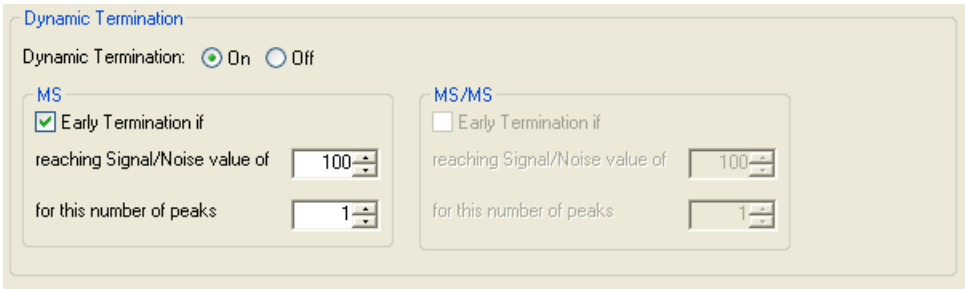


Figure 50 Activated Dynamic Termination feature

Movement tab

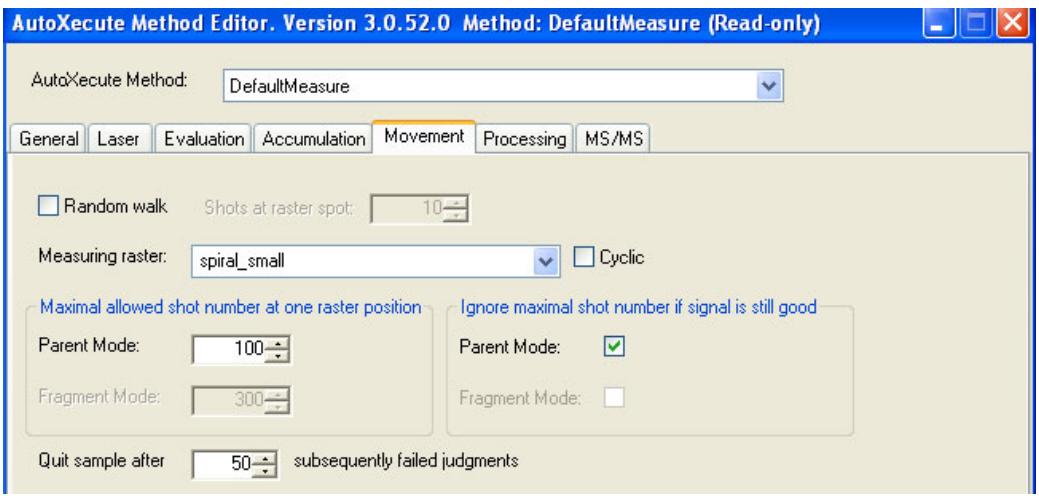

AutoXecute Method Editor. Version 3.0.52.0 Method: DefaultMeasure (Read-only)
AutoXecute Method: DefaultMeasure
General Laser Evaluation Accumulation Movement Processing MS/MS
Random walk
Shots at raster spot: 10
Measuring raster: spiral_small
Cyclic
Maximal allowed shot number at one raster position
Parent Mode: 100
Fragment Mode: 300
Ignore maximal shot number if signal is still good
Parent Mode:
Fragment Mode:
Quit sample after 50 subsequently failed judgments

Figure 51 Content of the *Movement* page

The ***Movement*** page (Figure 51) is used to specify whether the position on the sample spot (i.e. the raster spot) has to be changed, dependent on the sample quality. The different positions on a sample spot are named raster spot:

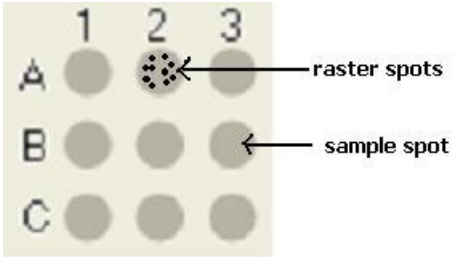


Figure 52 Sample spots and Raster spots

It is possible to measure a sample spot with a pre-defined pattern (**Measuring Raster**) or with the so called **Random walk**, where the laser fires on randomly chosen spots on the sample spot. All rastering features are de-activated in case of **Random walk**. To use this feature the number of shots per raster position has to be entered.

The **Random walk** is recommended for thin layer preparations (with and without Fuzzy control) and for the use with PAC targets.

If the **Cyclic** checkbox is activated and if all raster positions of one measuring raster have been used once, AutoXecute will restart with the first raster position.

When the **Maximal allowed shot number at one raster position** is fired, the target moves to the next measure position (raster spot) on the same sample spot to continue with the acquisition.

However, if the check box "**Ignore maximal shot number if signal is still good**" is activated the measurement will be continued as long as the evaluation considers spectra as good enough.

The **Maximal allowed shot number** and **Ignore maximal shots** can both be separately used for parents and fragments in an AutoXecute LIFT method.

The spin box **Quit sample after** is used to set a criterion to abort an acquisition on a sample spot. When the maximal number of subsequently failed judgments has been reached, the sample on the entire sample spot is considered too bad for data accumulation. The target moves to the next sample spot.

Processing tab

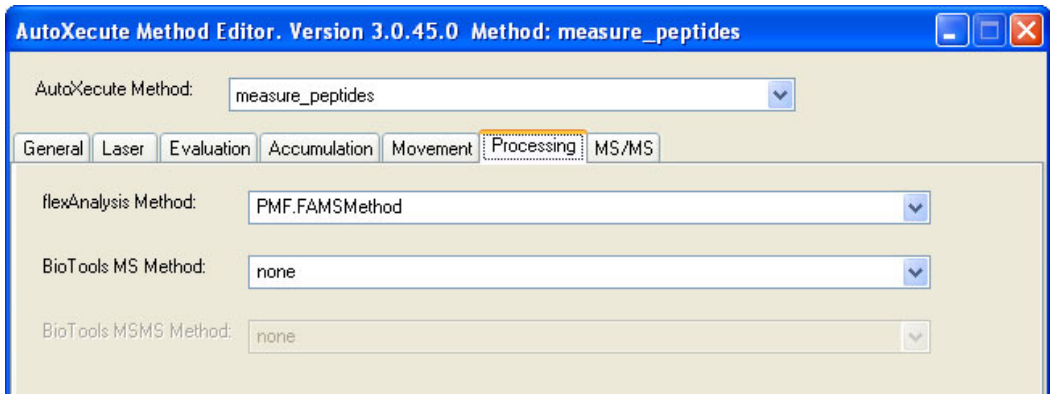


Figure 53 Content of the *Processing* page

The ***Processing*** page (Figure 53) is used to specify different available methods for post processing.

The combo boxes **flexAnalysis Method**, **BioTools MS Method**, and **BioTools MSMS Method** allow choosing one of the offered methods for post processing. If a method is chosen the method name is entered as information in the parameters of the saved spectrum (FAMeth and BTMeth). During an automatic run a spectrum is only sent to **flexAnalysis** and **BioTools**, if a respective method is assigned, either here in the AutoXecute Method Editor or via the Run Editor.

MS/MS tab

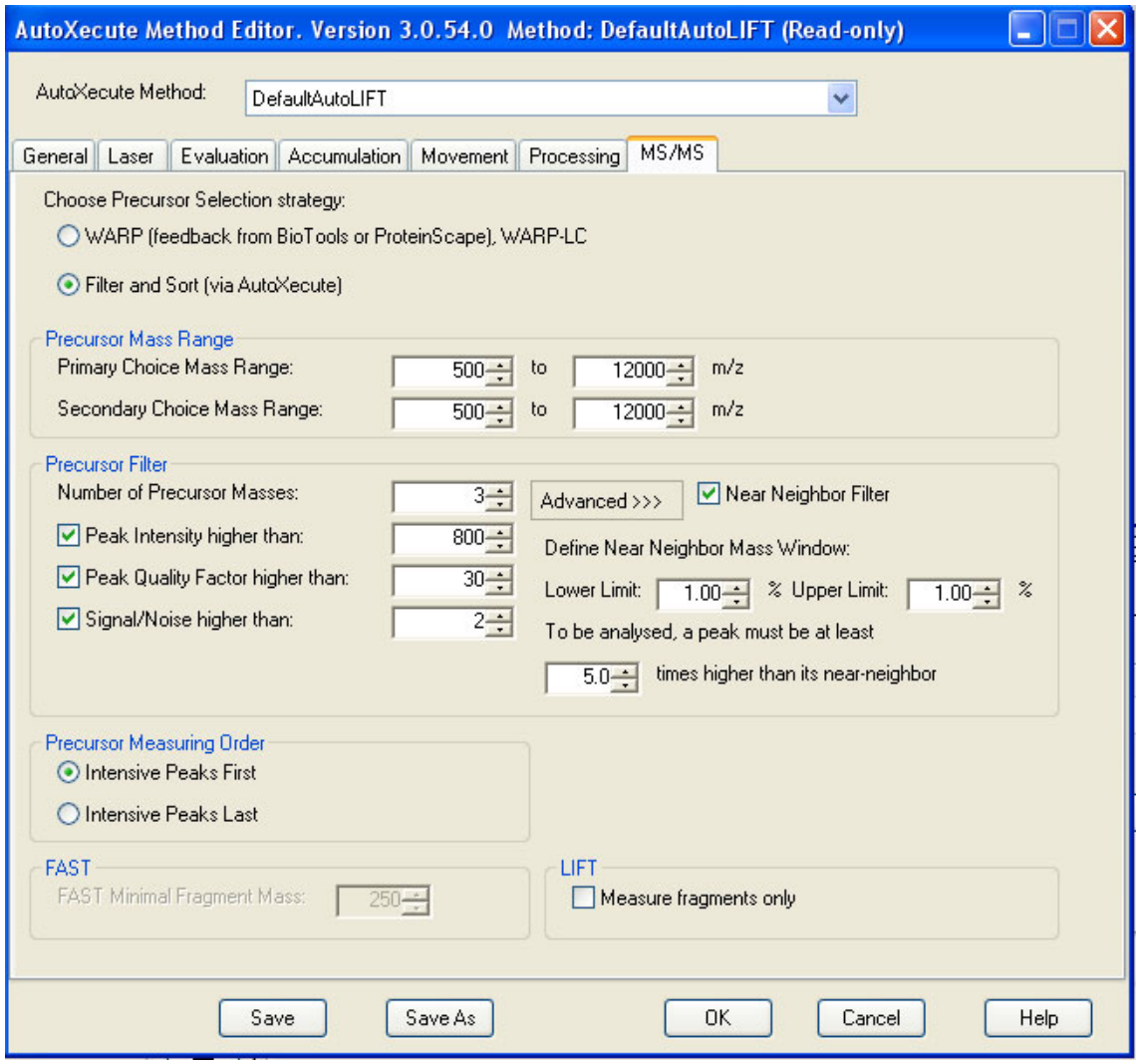


Figure 54 Content of the *MS/MS* page

If a **flexControl** LIFT- or FAST-method is loaded in the General tab, the MS/MS tab becomes activated. It is used to determine the LIFT and FAST measurements on the basis of the respective fingerprint information.

The main decision on this tab is done with the **Precursor Selection strategy**. For automatic MS/MS measurements it is essential to have MS fingerprint peaklists where special peaks are selected from. Two possibilities are offered to select these peaks from the MS peaklist:

1. **Warp – feedback from BioTools or ProteinScape**: BioTools (version 3.0 and higher) or ProteinScape mark peaks in the MS peaklist and sort this peaklist. AutoXecute then only reads this information and starts the MS/MS acquisition and evaluation without any further filtering. If **Warp** is chosen, the rest of the MS/MS tab is not used and therefore de-activated. WarpLC users have to use this precursor selection strategy. For more information see page A-I.
2. **Filter and sort – via AutoXecute**: AutoXecute reads the MS fingerprint peaklist and marks peaks for MS/MS, according to the filter criteria that are adjusted in the Precursor Filter group box. If this option is chosen, MS spectra can naturally also be sent to BioTools, but the Mascot result for these spectra does not affect the MS/MS measurement.

Due to the process of a MS/MS-measurement there is a mass range with optimal fragmentation properties. This behavior is addressed with ***Precursor Mass Range Selection***. Peaks in the **Primary Choice Mass Range** are preferred fragmentation targets. Only if there are too few peaks in the primary mass range after filtering, the peaklist is gradually filled up from the **Secondary Choice Mass Range**. So the primary mass range is always included in the secondary mass range.

All parameters contained in the group box ***Precursor Filters*** can individually be selected. They form a filter to ensure that only the best suitable precursors are taken for a measurement. The higher the values of the three parameters are set, the narrower is the bandwidth surrounding the precursor.

- Select the number of MS/MS measurements with **Number of Precursor Masses**. The masses that are selected will be listed in the AutoXecute output.
- With **Peak Intensity higher than** all peaks with lower intensity will be sorted out.
- With **Peak Quality factor higher than** all peaks with lower Quality factor will be sorted out.
- With **Signal/Noise high than** all peaks with lower S/N will be sorted out.

The information on the filtering during the run is shown in detail in the AutoXecute output. If you want to check the filter process you may load the MS spectrum manually in **flexAnalysis** and filter the peaklist step by step on your own.

In the *Advanced* section (Figure 55) a fourth filter, ‘Near Neighbor’, is offered with its belonging settings. It ensures that if peaks are in close proximity (ion selector window) of a possible precursor none of them is taken as precursor. The lower and upper limits define the mass window where no other peak must be detected. …

A valid precursor must have at least the defined intensity (regarding his near neighbors) to be analyzed.

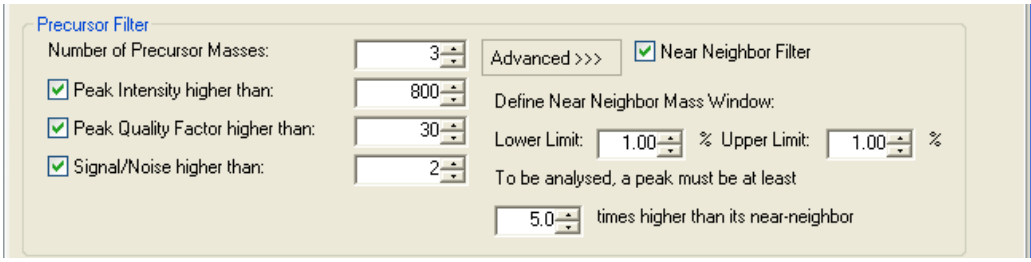


Figure 55 Precursor Filter - Advanced

Depending on the chosen radio button in **Precursor Measuring Order** the MS/MS measurement begins with the intensive or the small peaks first.

In case of an AutoXecute FAST measurement the minimal Fragment mass, that determines the number of segments, can be entered here and is transferred to **flexControl** during the run.

The LIFT option **Measure Fragments only** can be used e.g. for MALDI Imaging experiments. If you are interested, please have a look in the Imaging manual.

3.4.9.1.2 Run method on current spot

To test the settings of an AutoXecute method the

button can be used. Automatic data acquisition starts on the current spot with the currently selected AutoXecute method, but data are not saved automatically in the end. The **flexControl** method loaded in the GUI has to fit to the AutoXecute method. Otherwise a message appears in the AutoXecute output (Figure 56) and the acquisition does not start.

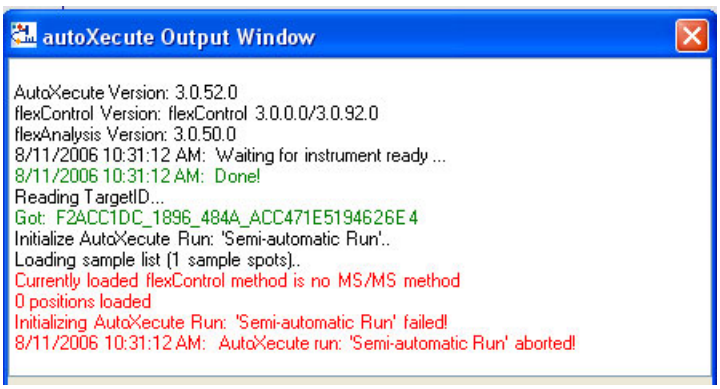

autoXecute Output Window
AutoXecute Version: 3.0.52.0
flexControl Version: flexControl 3.0.0.0/3.0.92.0
flexAnalysis Version: 3.0.50.0
8/11/2006 10:31:12 AM: Waiting for instrument ready ...
8/11/2006 10:31:12 AM: Done!
Reading TargetID...
Got: F2ACC1DC_1896_484A_ACC471E5194626E4
Initialize AutoXecute Run: 'Semi-automatic Run'..
Loading sample list (1 sample spots)..
Currently loaded flexControl method is no MS/MS method
0 positions loaded
Initializing AutoXecute Run: 'Semi-automatic Run' failed!
8/11/2006 10:31:12 AM: AutoXecute run: 'Semi-automatic Run' aborted!

Figure 56 AutoX output with error message

3.4.9.1.3 AutoX Run

AutoXecute runs (sequences) are created with the Run Editor that is part of **flexControl,** but can also be used as stand alone program. An **AutoXecute Run** defines the workflow on every prepared target spot, i.e. what to measure (MS, MS/MS), where to save the data and which post processing is done.

After an AutoXecute run is finished the measurement status for each spot has automatically been transferred to the sequence. It can be seen in the sequence file (Figure 59) as well as on the plate view (Figure 63). A run that has been finished cannot be started again without resetting the status. If you try to start an already finished run, the AutoXecute output gives the information that no measurement position is available (Figure 57). To run the sequence again the status for MS and/or MS/MS has to be reset in the Run Editor (Figure 59).

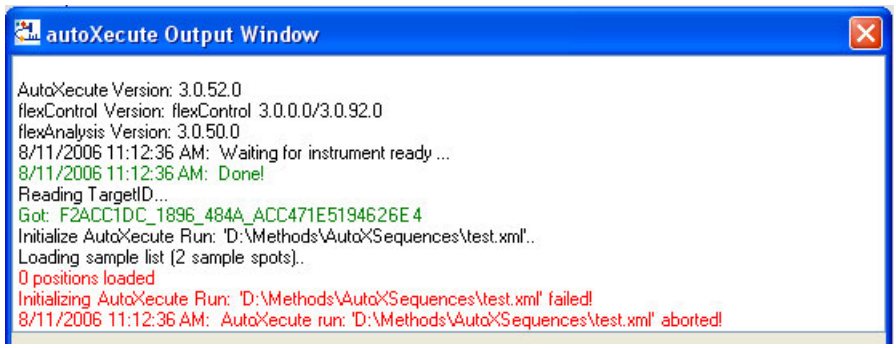
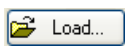
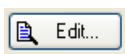

autoXecute Output Window
AutoXecute Version: 3.0.52.0
flexControl Version: flexControl 3.0.0.0/3.0.92.0
flexAnalysis Version: 3.0.50.0
8/11/2006 11:12:36 AM: Waiting for instrument ready ...
8/11/2006 11:12:36 AM: Done!
Reading TargetID...
Got: F2ACC1DC_1896_484A_ACC471E5194626E4
Initialize AutoXecute Run: 'D:\Methods\AutoXSequences\test.xml'..
Loading sample list (2 sample spots)..
0 positions loaded
Initializing AutoXecute Run: 'D:\Methods\AutoXSequences\test.xml' failed!
8/11/2006 11:12:36 AM: AutoXecute run: 'D:\Methods\AutoXSequences\test.xml' aborted!

Figure 57 A finished run has been re-started without resetting the status

A list of the last used sequences is offered in the drop down box **Run** (Figure 44). The number of sequences shown depends on the setting in the AutoXecute Settings dialog (Figure 64).

The button 

is used to load sequences that are not offered in the drop down list. It is also possible to load old sequence files (.txt) that have been created with the Excel based Spreadsheet Generator. It is not possible to load a run that was created for a target geometry that does not match to the target that is currently in the instrument. A message appears. To load this run, insert the correct target first.

The button 

is used to open the Run Editor with the currently selected sequence file. If a txt file (Spreadsheet Generator generated) is selected it will be imported, the appearing message (Figure 58) has to be confirmed. Methods, paths, sample names and comments will be transferred.

Figure 58 Import a Spreadsheet Generator created sequence

An AutoXecute Run is started with the button Start automatic Run. At the beginning the methods used in the run are checked. If a file is missing, the run stops and a message is given in the AutoXecute output.

During an AutoXecute run it is not possible to change any settings in either the AutoXecute methods or the sequence file. If you want to view a method, the run has to be paused with the button Pause Run from the output window or the AutoXecute tab. The Method Editor as well as the Run Editor can then be opened in the view mode where nothing can be changed. To continue click Resume Run.

3.4.9.1.4 The AutoXecute Run Editor

The button New... starts the Run Editor Wizard (if activated) or opens the Run Editor immediately (like the Edit... button does). If you start working with the Run Editor it is recommended to use the wizard. The explanations in the wizard and in the Help file of the Run Editor are very detailed, therefore not much additional information is given in this manual.

As denoted before, the status of a spot is shown in the sequence file. If the status is “finished” but you want to measure the spot again, it is necessary to **Clear the status** and save the sequence.

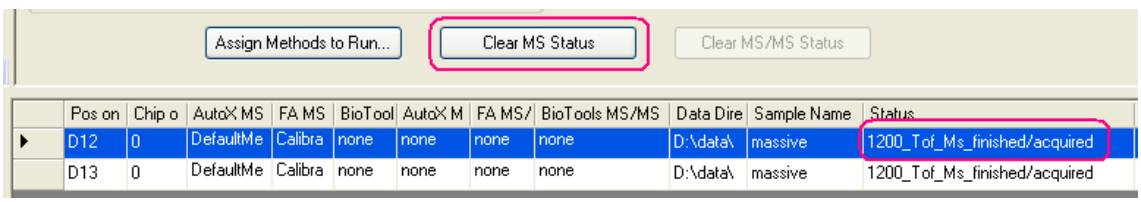
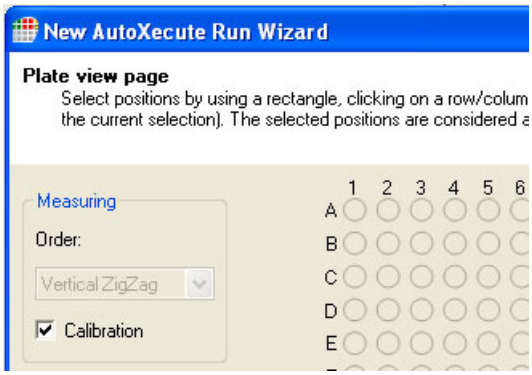


Figure 59 Status of a sample spot

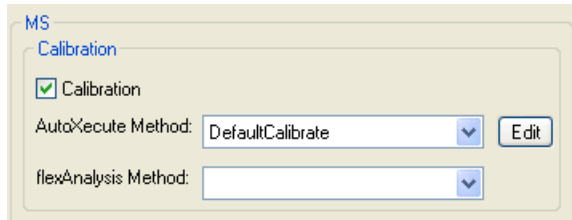
In earlier **flexControl** versions where sequences had to be created with the Spreadsheet Generator some templates were offered, especially for the calibration usecase. With the new Run Editor calibration templates are pre-installed for a lot of different target geometries. To be able to use a template, the **Calibration Mode** has to be activated in the **Run Editor wizard** or the **Run Parameter** page (see below).

According to the loaded geometry the matching calibration template is selected. With transfer of the spots to the sequence list (after method selection etc), spots of all available chips are inserted

Note: The calibration spots are pre-defined, so you have to take care to prepare the correct spots if you want to use the templates!



Calibration mode activated in the **wizard**.



Calibration mode activated in the **Run Parameter** page (main view of the **Run Editor**).

3.4.9.1.5 Show Output

Activating the **Show Output** checkbox opens the AutoXecute output window (Figure 60) where detailed information concerning the current autoXecute run is shown. In the beginning the complete version numbers of the programs are listed, afterwards follows the method check that assures that all selected methods from the run are available on the hard drive.

During the run a lot of information concerning laser power setting, peak evaluation etc are displayed. If errors occur they will be shown in red, successful evaluations are shown green colored. More information on how to interpret the fuzzy engine results (fuzzy input/output) are given in the pdf "*How to read AutoXecute log.pdf*" on the Compass for flex CD.

A run can be paused and continued with the button Pause Run / Resume Run and aborted with Abort Run. Information on these actions are shown in the output.

The content of the AutoXecute Output Window is also saved on the hard drive in the file C:\BDALSystemData\AutoXOutput.

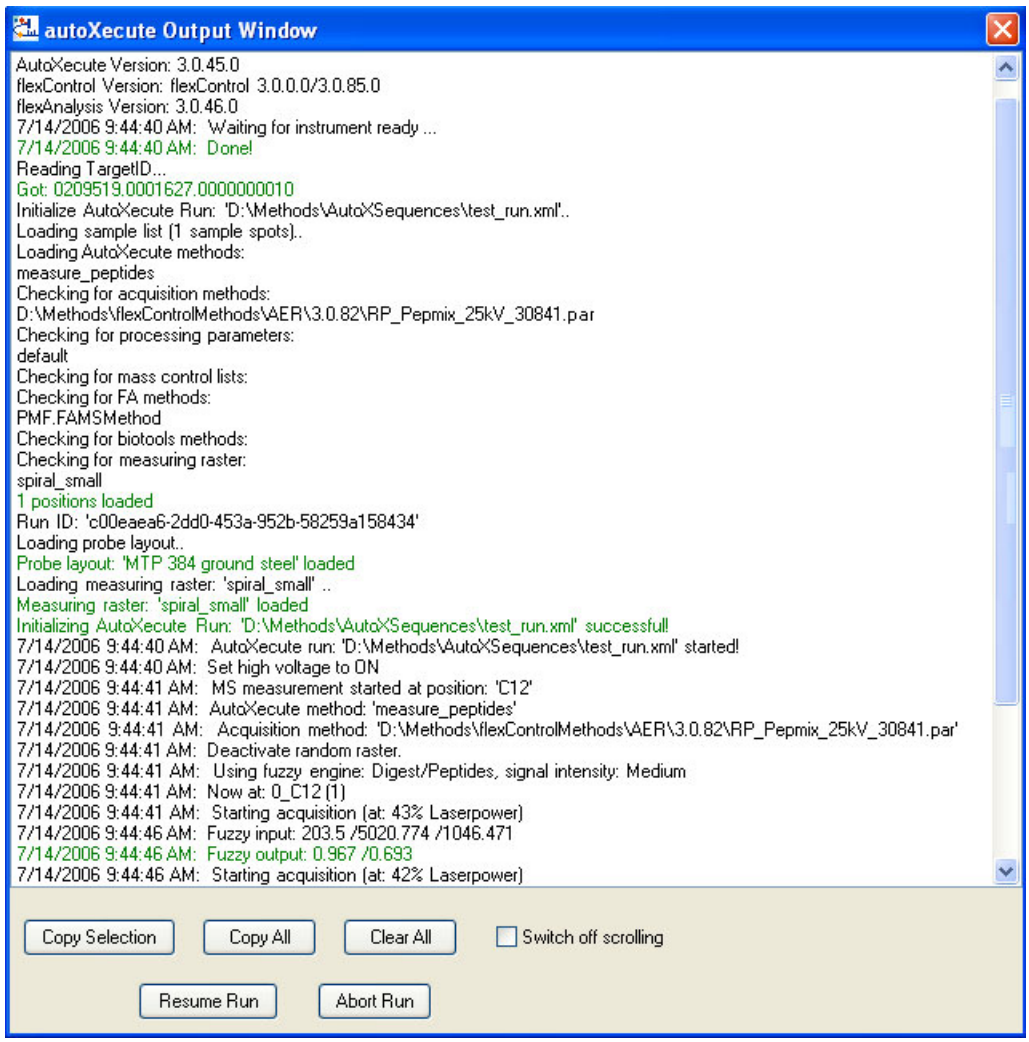

autoXecute Output Window
AutoXecute Version: 3.0.45.0
flexControl Version: flexControl 3.0.0.0/3.0.85.0
flexAnalysis Version: 3.0.46.0
7/14/2006 9:44:40 AM: Waiting for instrument ready ...
7/14/2006 9:44:40 AM: Done!
Reading TargetID...
Got: 0209519.0001627.0000000010
Initialize AutoXecute Run: 'D:\Methods\AutoXSequences\test_run.xml'..
Loading sample list (1 sample spots)..
Loading AutoXecute methods:
measure_peptides
Checking for acquisition methods:
D:\Methods\flexControlMethods\AER\3.0.82\RP_Pepmix_25kV_30841.par
Checking for processing parameters:
default
Checking for mass control lists:
Checking for FA methods:
PMF.FAMSMethod
Checking for biotools methods:
Checking for measuring raster:
spiral_small
1 positions loaded
Run ID: 'c00eaea6-2dd0-453a-952b-58259a158434'
Loading probe layout..
Probe layout: 'MTP 384 ground steel' loaded
Loading measuring raster: 'spiral_small' ..
Measuring raster: 'spiral_small' loaded
Initializing AutoXecute Run: 'D:\Methods\AutoXSequences\test_run.xml' successful!
7/14/2006 9:44:40 AM: AutoXecute run: 'D:\Methods\AutoXSequences\test_run.xml' started!
7/14/2006 9:44:40 AM: Set high voltage to ON
7/14/2006 9:44:41 AM: MS measurement started at position: 'C12'
7/14/2006 9:44:41 AM: AutoXecute method: 'measure_peptides'
7/14/2006 9:44:41 AM: Acquisition method: 'D:\Methods\flexControlMethods\AER\3.0.82\RP_Pepmix_25kV_30841.par'
7/14/2006 9:44:41 AM: Deactivate random raster.
7/14/2006 9:44:41 AM: Using fuzzy engine: Digest/Peptides, signal intensity: Medium
7/14/2006 9:44:41 AM: Now at: 0_C12 (1)
7/14/2006 9:44:41 AM: Starting acquisition (at: 43% Laserpower)
7/14/2006 9:44:46 AM: Fuzzy input: 203.5 /5020.774 /1046.471
7/14/2006 9:44:46 AM: Fuzzy output: 0.967 /0.693
7/14/2006 9:44:46 AM: Starting acquisition (at: 42% Laserpower)
Copy Selection
Copy All
Clear All
Switch off scrolling
Resume Run
Abort Run

Figure 60 AutoXecute Output

3.4.9.1.6 Settings

The Settings... button opens the AutoXecute Settings dialog (Figure 61) that helps to configure some general AutoXecute settings that do not need to be changed for every run.

Note: After changes have been done in this dialog **flexControl** has to be re-started to be able to work with the new settings!

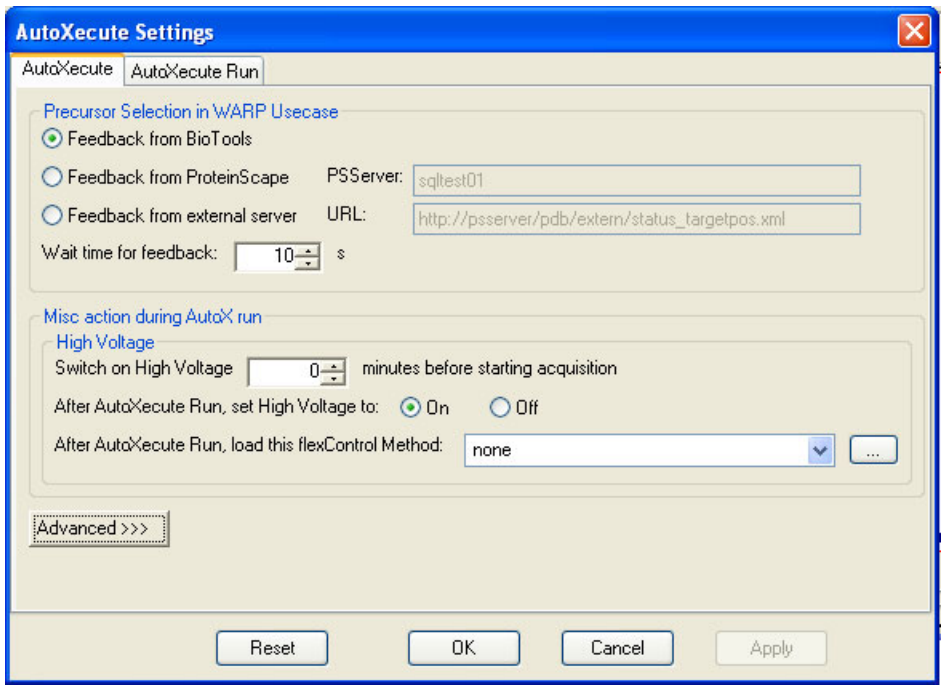


Figure 61 AutoXecute Settings – AutoX

Precursor Selection in WARP Usecase

If the WARP usecase is selected in the MS/MS AutoXecute method (page 3-55), an external program like BioTools or ProteinScape selects the masses for later MS/MS measurement. In this dialog the respective **Feedback**-radiobutton is chosen to determine to work with either of these programs.

The **Wait time** applies only for BioTools. It specifies how long AutoXecute waits for the modified fingerprint peaklist. If after this time no peaklist is available for the current sample spot, AutoXecute continues with the next spot. Since common AutoXecute runs consist of more than a few spots, and first all MS measurements should be done,

BioTools or ProteinScape will normally have enough time to modify the MS peaklists. So, if AutoXecute starts with MS/MS the MS information are available.

If ProteinScape has not been finished when AutoXecute starts with MS/MS the run stops immediately without any wait time.

Note: In case BioTools or ProteinScape are not ready when AutoXecute wants to start the MS/MS measurement, the run ends without MS/MS. Afterwards, when the necessary modifications from BioTools or ProteinScape are available, i.e. peaks are marked, it is possible to re-start the run with measure MS/MS only.

Misc action during AutoX runs

The group box is used to define a period of time, the high voltage is switched on before starting the acquisition to put the instrument into a stable state. After a complete run the high voltage might be turned off with the corresponding radio button. This can be done over a weekend when the instrument is not in use.

Additionally an acquisition method may be loaded to prepare the mass spectrometer for the next run. If “none” is chosen for the **flexControl** method after a run, the last method loaded during the run remains in **flexControl**.

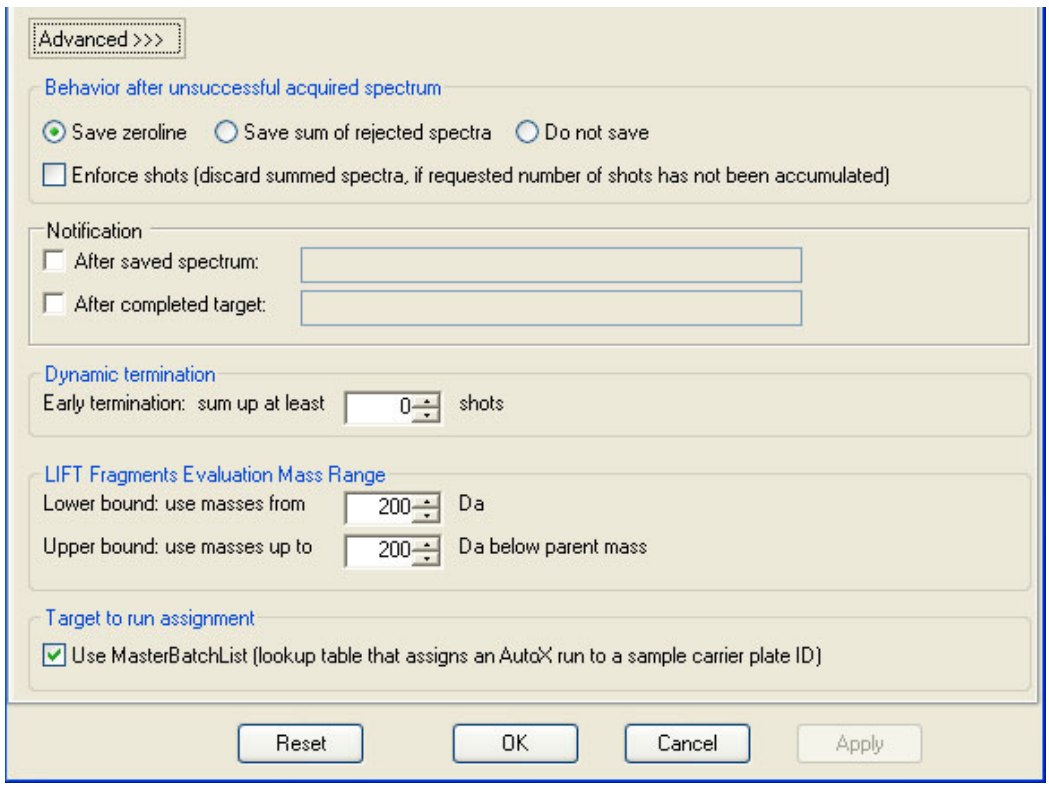
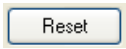


Figure 62 AutoXecute Settings – AutoX - Advanced

The *Advanced* section contains settings that should not be changed without exact knowledge (if questions occur, ask maldi.sw.support@bdal.de). If changes have been done but you are not sure, the pre-defined settings can be restored with the button 

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Behaviour after unsuccessful acquired spectrum

Different save results are achieved, depending on the combination of the (in-)activated checkbox **Enforce Shots** and the **Save** radio button. The results of the combinations are displayed in the following two tables. The colors correspond to the colors shown on the plate view (Figure 63) after the run has been finished

1. **Enforce Shots** is activated (i.e. the requested number of successfully added shots has to be reached)

	Save zeroline	Save sum of rejected	Do not save
No shot package is summed	Zeroline	Zeroline	Nothing saved
Some shot packages are summed	Zeroline	Zeroline	Nothing saved

2. **Enforce Shots** is *not activated*, i.e. it is not necessary to have all requested shots in the end. Therefore you have to differentiate between ‘some shots have been added’ and ‘no shots have been added’:

	Save zeroline	Save sum of rejected	Do not save
No shot package is summed	Zeroline	Sum of rejected	Nothing saved
Some shot packages are summed	Sum of successfully added	Sum of successfully added	Sum of successfully added

The plate view may look as follows:

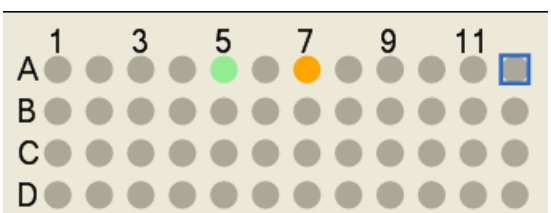


Figure 63 Plate View with colored results

Notification

After a spectrum has been saved or the measurement on a whole target is finished it is possible to get a notification on another server.

Dynamic Termination

This setting concerns the Early Termination feature from the Accumulation tab in the AutoXecute Method Editor (Figure 50), where it is possible to finish the accumulation on a sample spot if a defined number of peaks have reached a defined Signal/Noise value. Here you can specify the number of shots that has to be added at least before the Early Termination feature is allowed to end the acquisition as soon as the other criteria are reached.

LIFT Fragments Evaluation Mass Range

During the automatic measurement of LIFT *fragment* spectra these values determine the respective mass range where peaks are used from for evaluation.

Target to run assignment

Activate this checkbox, if you want to use the MasterBatchList. Normally this is only used in conjunction of the Twister use.

History Count

The **History Count** determines the number of runs that is offered in the drop down box **AutoX Run** in Figure 44.

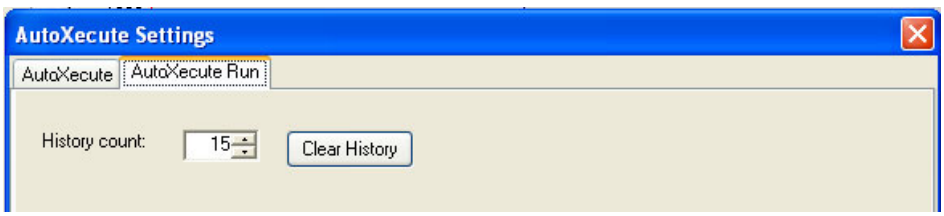


Figure 64 AutoXecute Settings – AutoX Run

3.4.9.2 Sample Carrier

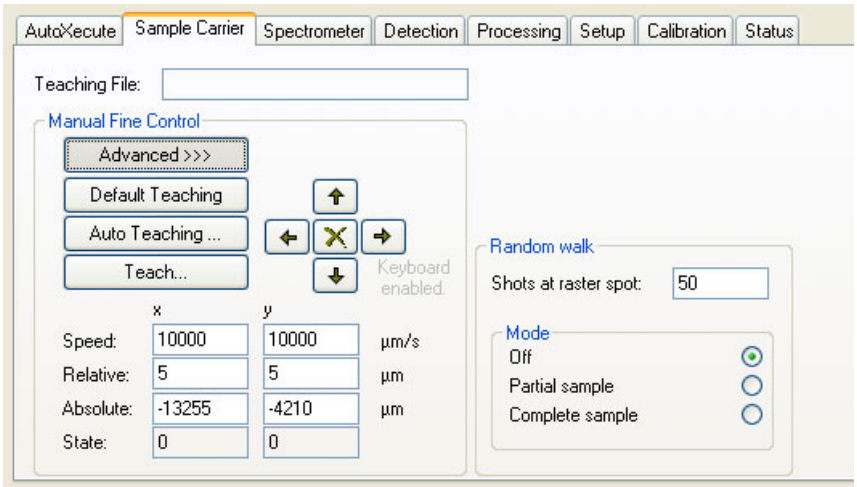


Figure 65 Features of the *Sample Carrier* page

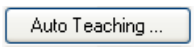
The ***Sample Carrier*** page (Figure 65) is used to configure target plate features. On the top the Teaching File is shown that belongs to the currently loaded target geometry (Figure 38).

The button

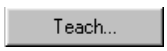
opens the group box 'Manual Fine Control' on the right. Clicking the symbol in the center of the arrangement of arrows activates the keyboard cursors to move the tray. This option allows a sharp and exact move across a sample spot. A second way to alter a position on the spot is to enter corresponding values of the x-y-parameters entry fields on the right.

The

button loads a teaching file (<name>.**fact**) corresponding to the currently loaded target plate. These files are made at the factory for all the kinds of target plates the drop down list **Geometry** contains.

The button 

starts the auto-teaching process (for details see section 3.4.9.6). If no auto-teaching target is in the instrument a message appears and the process stops.

The button 

opens the teaching dialog.

3.4.9.2.1 Teaching Dialog

Teaching is an alignment procedure to compensate possible mechanical tolerances of the sample loading facility.

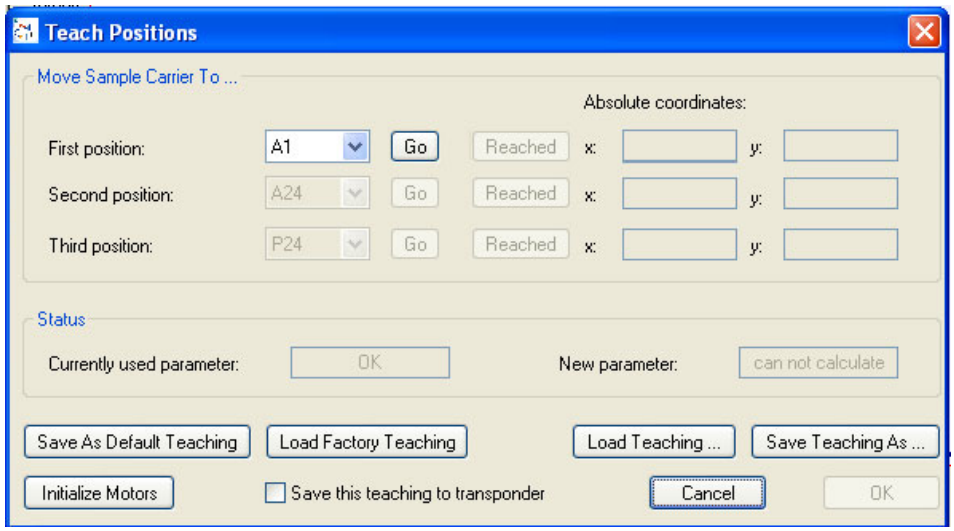


Figure 66 Features of the teaching dialog

The dialog shown in Figure 66 appears for realignment. To perform a teaching select the **First Position** and click on Go. When the target has been moved to this position, click Reached and continue with the **Second Position**. The new absolute coordinates will be calculated automatically.

When the group box 'Status' acknowledges with OK quit with the OK button. The record of the new x-y-coordinates is finished and sent to the transponder in case of transponder targets, if the checkbox "Save this teaching to Transponder" is activated.

Use the button Initialize Motors before teaching to eliminate possible step losses. The task of the button is to re-define the zero point of the x-y-coordinates. The button Load Factory Teaching loads a file containing factory defaults of the target. The buttons Load Teaching ... and Save Teaching As ... are used to handle <**name**>.teach-files, either to re-load a stored file or to store a current file with a specific name.

3.4.9.2.2 Random Walk

The **Random Walk** feature can either be used during manual measurement but also during automatic data acquisition (page 3-52). It allows to measure on randomly selected raster positions.

Select Mode ***Off*** if you want to choose the measuring positions on your own.

If you activate ***Partial Sample,*** only a small area around the actual chosen raster position in the video is used for data acquisition.

Note: in automatic mode partial sample cannot be used – complete sample is used instead if the random walk is activated.

With ***Complete Sample*** selected the laser shots on the whole sample spot. **Shots on a raster spot** determine the number of shots that is fired on every randomly selected position.

3.4.9.3 Spectrometer

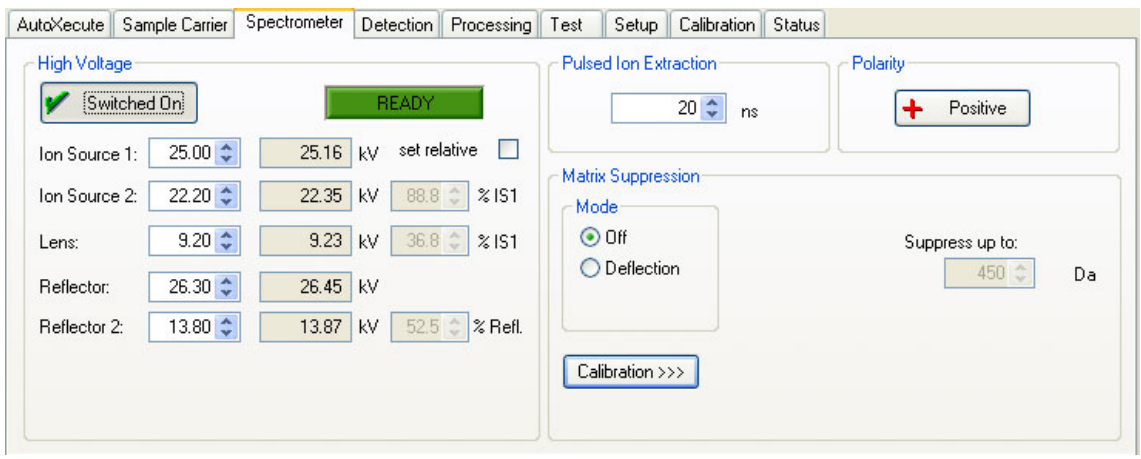
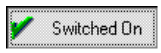


Figure 67 Features of the *Spectrometer* page in case of a reflector method

The ***Spectrometer*** page (Figure 67) is used to configure the ion optics. According to the loaded method this page offers specific features.

The toggle key  | is used to switch on or off following components, depending on the loaded method:

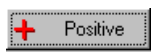
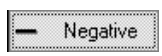
- Ion Source 1,
- Ion Source 2,
- Lens,
- Reflector,
- Reflector 2,
- LIFT,
- LIFT 2.

The status field / right beside the | button is the corresponding feedback, whether the HV-supply is ready or not. In case of a bad vacuum, or when the instrument is vented this field toggles to (high voltage is definitely **off**) independent of the switch position.

Below the toggle buttons are edit fields to adjust voltages for the target plate (ion source 1), the electrode P2 (ion source 2), and the lens arrangement. The grayed fields reflect the actual measured values for diagnostics. They may deviate and fluctuate, as threshold values are not very accurate.

Selecting the box “set relative” activates the two spin boxes “**%IS1**”. The upper spin box is used to adjust the IS2 value relative to IS1. The other spin box is used to set the lens voltage relative to IS1. These three adjustments may be varied to get good results in resolution and sensitivity.

The value for ‘Pulsed Ion Extraction’ is the delay measured in ns between the laser shot and the moment when the electrode P2 is pulsed down to let the ions pass.

The toggle button  |  allows to acquire positive or negative ions.

Matrix Suppression is a feature useful for suppressing matrix components of a compound and avoiding saturation effects of the detector. If the radio button “Off” inside the group box ‘Mode’ is not applied either the button “Deflection” or the button “Gating” (available in the **linear** mode) becomes active.

The matrix deflection process is performed inside the ion source by coupling a pulse to the related deflection plate. The pulse length corresponds to the mass entered into the field **Suppress up to**. The mass entered here is the lowest displayed mass in the spectrum.

The gating process occurs inside the linear detector by applying fractions of the supply voltage for few µs to the first channel plate of the detector. A couple of ms later on the voltage returns back to its nominal value.

flexControl detects instruments that do not have an ion source with deflection electrodes, such as the **microflex**. The program automatically uses for deflection either the PCIS or PLMS (tandem spectrometers) electrode.

Setting a second reflector voltage (Reflector 2) is required using an **ultraflex TOF/TOF** spectrometer.

If the checkbox “set relative” is activated the second reflector voltage can be adjusted in the spin box “**%Refl.**” as a percentage of the first reflector voltage. Concerning the **Matrix Suppression** feature only the **Deflection** option is applicable.

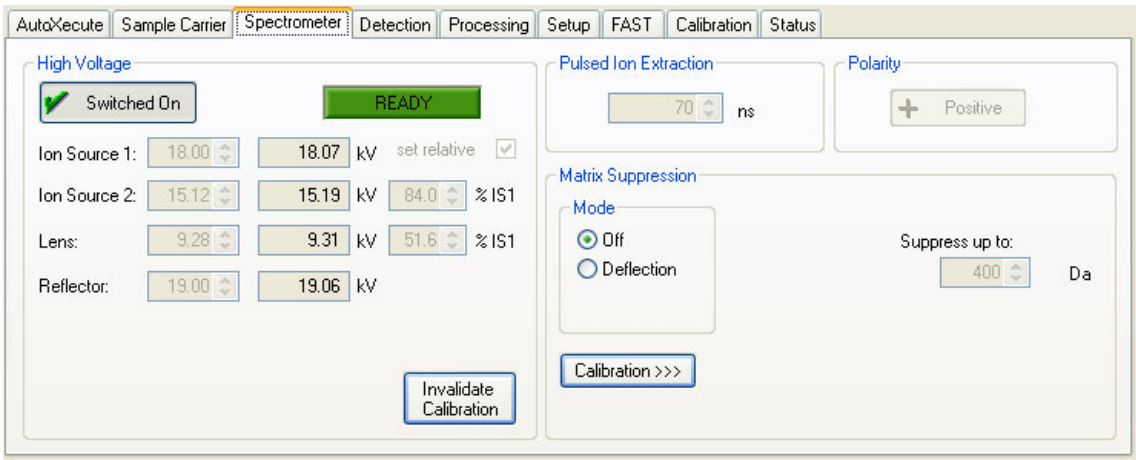


Figure 68 Features of the *Spectrometer* page in case of a FAST-method (microflex)

Loading a valid FAST method deactivates the access to all the entry fields. If the user wants to change voltage settings, he has to apply the Invalidate Calibration button. Then the message below appears.

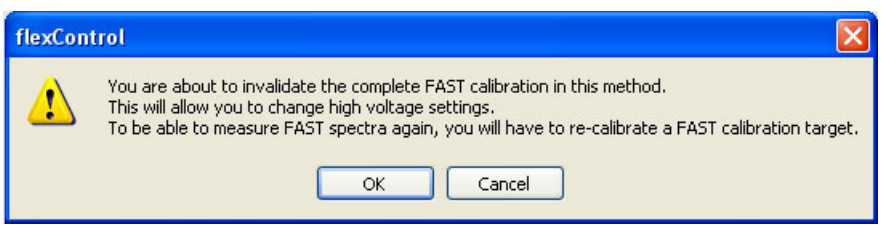

flexControl
You are about to invalidate the complete FAST calibration in this method.
This will allow you to change high voltage settings.
To be able to measure FAST spectra again, you will have to re-calibrate a FAST calibration target.
OK
Cancel

Figure 69 Warning

Applying the “OK” button releases the edit fields for new voltage adjustments. This action requires a new FAST calibration.

In case of a tandem mass spectrometer and a loaded LIFT-method the content of the ***Spectrometer*** page changes as shown in Figure 70. Compared to the previous example shown in Figure 67 the voltages for the additional two LIFT-electrodes have to be adjusted.

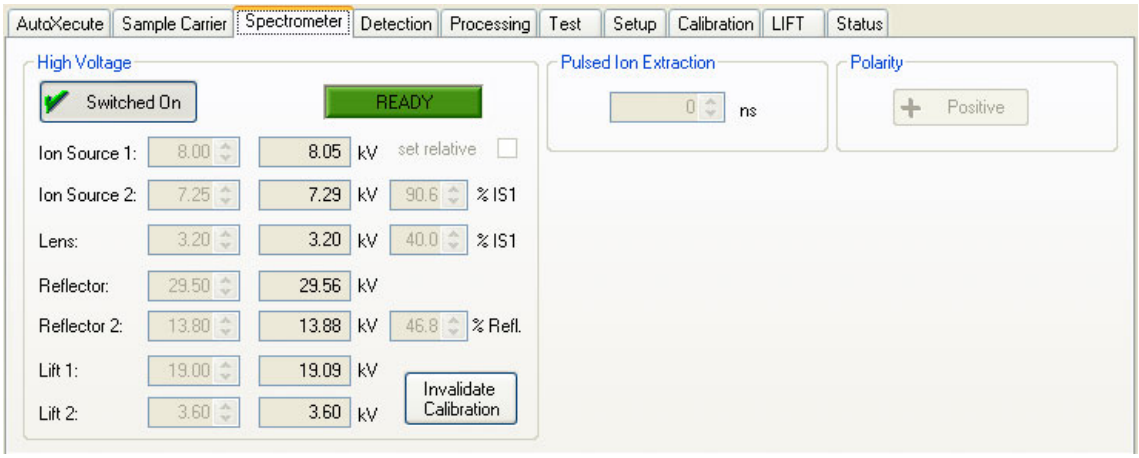


Figure 70 Features of the *Spectrometer* page in case of a LIFT-method

Loading a valid LIFT method deactivates the access to all the entry fields. If the user wants to change voltage settings, he has to apply the Invalidate Calibration button. Then the message shown in Figure 71 appears.

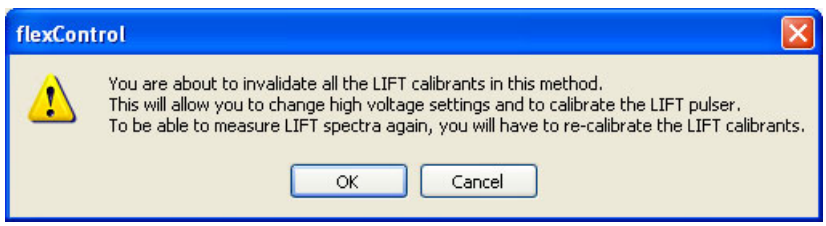

flexControl
You are about to invalidate all the LIFT calibrants in this method.
This will allow you to change high voltage settings and to calibrate the LIFT pulser.
To be able to measure LIFT spectra again, you will have to re-calibrate the LIFT calibrants.
OK
Cancel

Figure 71 Warning

Applying the “OK” button releases the edit fields for new voltage adjustments. This action requires a whole new LIFT calibration described in section 3.4.9.9.

3.4.9.4 Detection

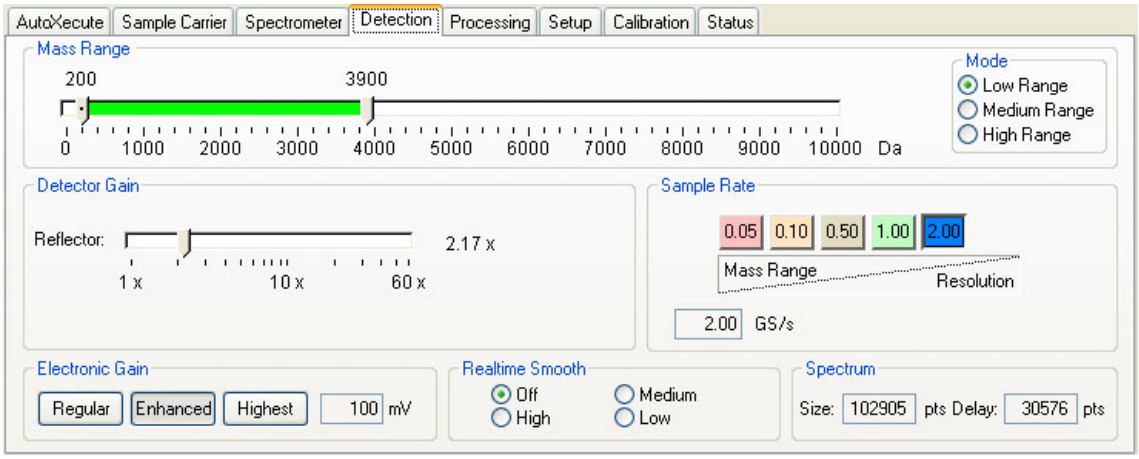


Figure 72 Features of the *Detection* page (microflex)

The ***Detection*** page (Figure 72) is used to readjust acquisition parameters, e.g., to configure the mass scale, or to vary the amplification factor of the detector.

The total mass range of 500.000 Da is divided into three sub ranges called **Low Range**, **Medium Range,** and **High Range**. Anyone can be independently configured according to the application. This configuration comprises the definition of the start and end mass and the linear or logarithmic scaling of the range or sub ranges.

To configure a mass range press the button

on the ***Setup*** page (section 3.4.9.6).

Sometimes the S/N ratio of the acquired data requires an adaptation of the detector gain. The slider is used to adapt the amplification factor. The function of the slider corresponds to that of the edit field **Reflector Detector Gain** in the **Fast Method Editor** (3.4.9.11.1). A change made in one of both features is simultaneously adopted by the other one.

The parameters *Resolution* and *Mass Range* of the group box 'Sample Rate' correlate in a way that an increase of the Sample Rate causes an increase of the resolution and a decrease of the mass range (digitizer performance). To illustrate this the invalid part of the mass range selector becomes red in color.

The group box 'Electronic Gain' contains buttons to select the amplification factor of the digitizer. The selected factor is part of the **flexControl** method, but the respective values are defined in the Instrument Settings file <name>.isset (section 3.4.9.6).

"Realtime Smooth" reduces the bandwidth of the digitizer preamplifier. Depending on your digitizer type the ***Detection*** page either contains a checkbox to simply switch this

feature on or off, or **flexControl** provides radio buttons to adjust three different bandwidths, or to switch smoothing off. This function is the same as that selectable by the **Fast Method Editor** (section 3.4.9.11.1).

The fields in the group box 'Spectrum' are read only fields. They show the size of spectra in points. Delay refers to the start delay of the digitizer. The displayed sample steps are not recorded.

An autoflex mass spectrometer may be equipped with a **High Mass** detector instead of the standard MCP detector (**M**ulti **C**hannel **P**late). Operating in linear mode (linear method is loaded) together with a High Mass instrument additional High Mass parameters appear in the ***Detection*** page (Figure 73). High Mass detectors have an additional acceleration electrode, which operates best at a voltage of 8 - 10kV. The task of this electrode is to accelerate secondary electrons.

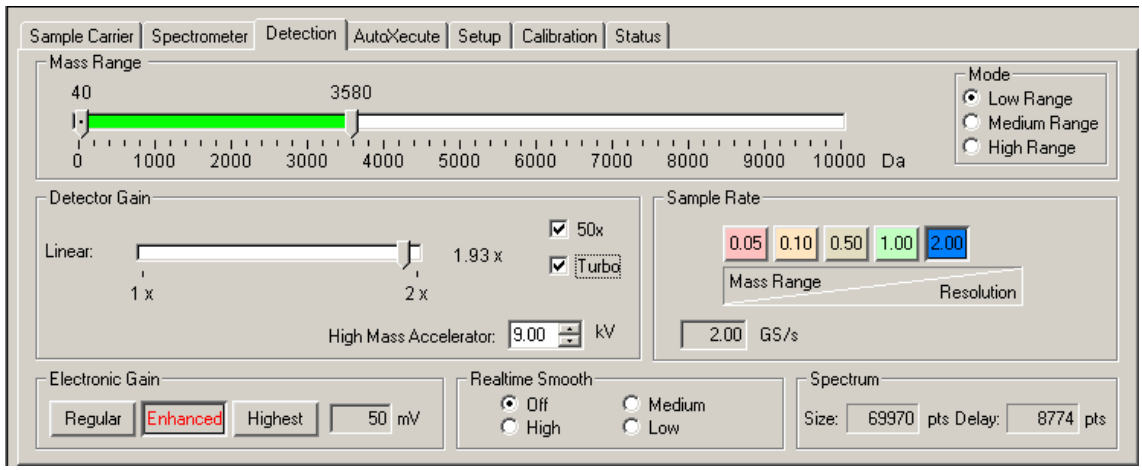


Figure 73 Features of the *Detection* page if an autoflex uses a Highmass detector

The checkbox "50x" is used to increase the input sensitivity of the digitizer.

The checkbox "Turbo" adapts the detector sensitivity for masses beyond 10kDa (polymers). It is on the operator to experiment with these settings for the best performance.

3.4.9.5 Processing

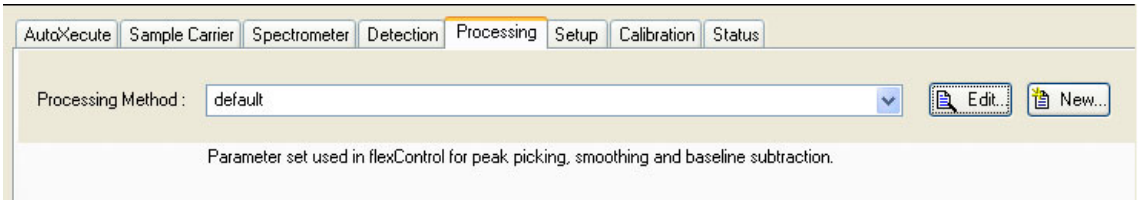


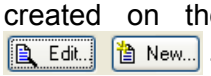
Figure 74 The *Processing* page

The ***Processing*** page (Figure 74) offers access to the Processing Parameter Methods that contain the currently active peak picking and processing settings. Processing Methods (*.prp) are used, created and changed from **flexControl**, **flexAnalysis** and **AutoXecute**. If a Processing method is edited in one of these programs the changes are immediately available in the other programs.

A Processing method is part of the **flexControl** method in a way that the **flexControl** method contains a reference to a Processing method. If a new Processing method is selected, this is detected as change of the FC method that has to be saved then. If a parameter in the current Processing method is changed only the Processing method has to be saved, the FC method does not notice this change.

The Processing method that is loaded with a **flexControl** method defines the peak picking algorithm that is used for manual calibration and annotation in the display, as well as the algorithms and parameters that are used for manual baseline subtraction and smoothing.

When flexControl methods created and used with a former flexControl version are loaded the first time in flexControl 3.0, a processing method is automatically created and the old processing parameter are transferred into a Processing method. The new parameter gets the name of the flexControl method. For more information see the *Quickstart_ProcessingParameter.pdf* on the Compass for flex CD.

Existing Processing methods can be edited and new Processing methods can be created on the basis of the pre-installed *default* method. With the buttons 

the Processing Method Editor (Figure 75) opens. The section *Mass List – Find* covers the settings for the peak picking algorithm. Smoothing and Baseline Subtraction can be adjusted in the *Processing* section. The section *Mass List – Edit* is not relevant for **flexControl** and **AutoXecute**, but only for **flexAnalysis**.

Note: The Processing settings used in **flexControl** during the data acquisition are not saved with the spectrum and are not automatically available in **flexAnalysis** afterwards. This parameter set is only used in **flexControl**. The assignment of special Processing parameters to a spectrum has to be done via the **flexAnalysis** method:

1. Create a **flexAnalysis** method with a reference to your special Processing Parameter method (see **flexAnalysis** manual).
2. Acquire a spectrum in **flexControl** and open the “Save As” dialog (Figure 10).
3. Select the special **flexAnalysis** method (Process with) and save the spectrum.
4. The spectrum opens in **flexAnalysis** with exactly the assigned processing parameter.

For data acquisition in **flexControl** and evaluation during automatic runs the recommended peakfinder is Centroid. Using SNAP will only extend the processing time but not improve the spectra quality. SNAP should be used later on in **flexAnalysis** for creating peaklists.

Three different smoothing algorithms are offered. Which one is used depends on the application and on the user’s experience.

The baseline subtraction algorithm **ConvexHull** is usually preferred for a mass range of up to 10 kDa. The advantage of this algorithm is that the baseline is a smooth function. **TopHat** should not be used for Protein spectra.

For detailed information on the peak picking, smoothing and baseline subtraction algorithms please have a look in the **flexAnalysis** manual.

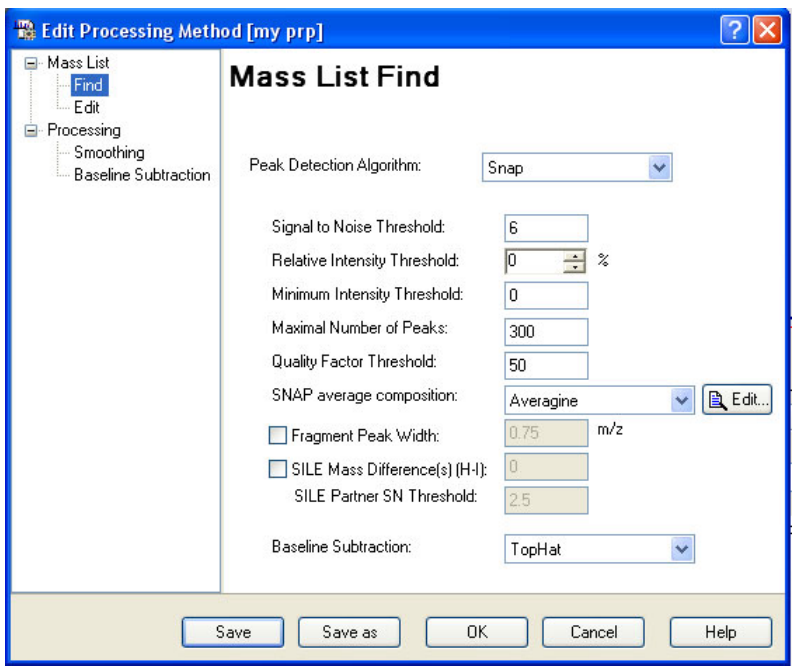


Figure 75 Processing Parameter Editor

3.4.9.6 Setup

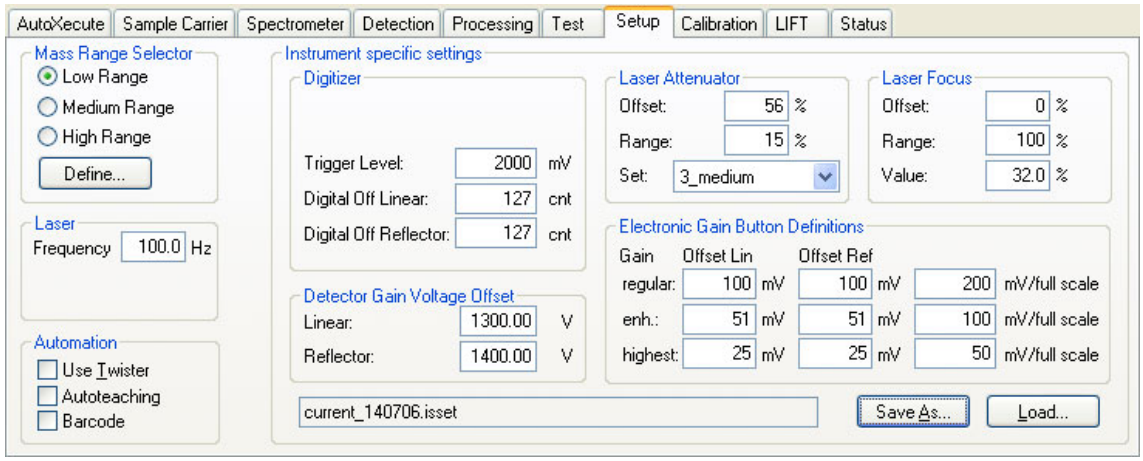
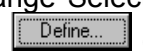


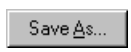
Figure 76 Features of the *Setup* page

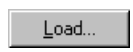
The ***Setup*** page (Figure 76) is used to adjust peripheral equipment, such as detector, laser attenuator and digitizer, etc. Depending on the *Bruker Daltonics UserManagement* given rights this tab is visible or invisible.

- Group box **Mass Range Selector**: The function Mass Range Selection is the same as that in the *Detection* page (3.4.9.4). The button 

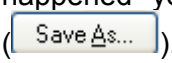
opens the dialog “Mass Range Selector” covered in section 3.4.9.7.
- Group box **Laser**: This field is used to adjust the shot frequency of the laser. Arbitrarily chosen values will be automatically changed to the next valid value. New entries are shown at the same time in the field **Frequency** in the **Acquisition Control Segment** (Figure 37).
- Group box **Automation**: It can contain three checkboxes, depending on the available hardware. Selecting the “**Use Twister**” box activates the Twister. This is a robotic micro plate loading system mounted in front of the ultraflex, or the autoflex mass spectrometer. It transports sample micro plates, such as the SCOUT MTP Target Plate from an input rack to the mass spectrometer. After the measurement the mass spectrometer returns the target to the Twister, which deposits it into the output rack.
- **Autoteaching** is an alignment procedure to automatically guide AnchorChip targets after docking into the best measure position. Autoteaching is performed whenever the target plate is changed, and if the target is identified as an AnchorChip type.

- Selecting the checkbox **Barcode** enables the barcode reader. If selected, Barcode identification has priority over transponder identification. Target plates without barcodes are identified by the IDs stored in their transponders.
- Group box **Instrument Specific Settings**: This box allows adjusting instrument settings, which are not performed in **flexControl** methods, e.g., for the digitizer.
- Group box **Digitizer**: The digitizer is equipped with two input channels. One is coupled to the linear detector the second is used for the reflector detector. The values entered in **Digital Off Linear** (**Reflector**) correspond to a number of counts, which is continuously subtracted from the signal level to fix the baseline to zero during a scan sequence.
- Group box **Laser Attenuator**: The edit fields are used to define the operating range of the laser slider on the **Acquisition Control Segment** (Figure 37). Normally the values entered in **Offset** and **Range** fix the bounds of a subrange within the range of the total available laser power. This restriction allows a finer adjustment of laser power in this portion. In this example the value entered in **Offset** is the lower bound and initial value within this confined operating range of the slider, which corresponds to 50% of the total available laser power. The entered value in **Range** (20%) corresponds to the upper bound of the slider's operating range, which is 50% of the total available laser power. Within this confined range the (relative) laser power can be varied from 0 % to 100 %.
- Group box 'Laser Focus': This function is only active in case of a REFLEX and an ultraflex mass spectrometer. The values for **Offset** and **Range** define also an operating range and **Value** focuses a lens into the optimal position within this predefined range.
- Group box 'Detector Gain Voltage Offset': The two reflector voltages correspond to a bias where the amplification factor of each detector is assumed These values may be adjusted to the experiment. Additionally the voltage for the reflector detector may be changed with the corresponding slider in the ***Detection*** page (3.4.9.4).
- Group box 'Electronic Gain Button Definitions': Each input channel of the digitizer has three sensitivity levels. The entered value here avoids that the baseline of the acquired spectrum moves below the zero line.

The functions of 

/ 

allow to save suitable instrument settings with a new name (<**name**>.isset) and to reload previously saved setting files.

If you want to exit **flexControl** it may happen that a message appears that the instrument specific settings have changed (Figure 77). If you are not sure what happened you may click on "Cancel" and save the settings with a new name (

). Afterwards **flexControl** can be closed.

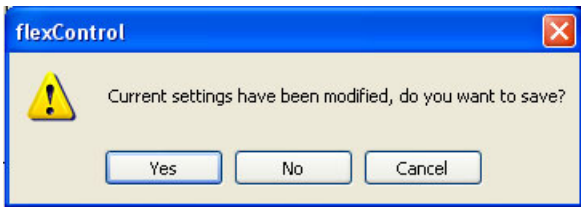


Figure 77 Instrument settings have changed

3.4.9.7 Mass Range Selector

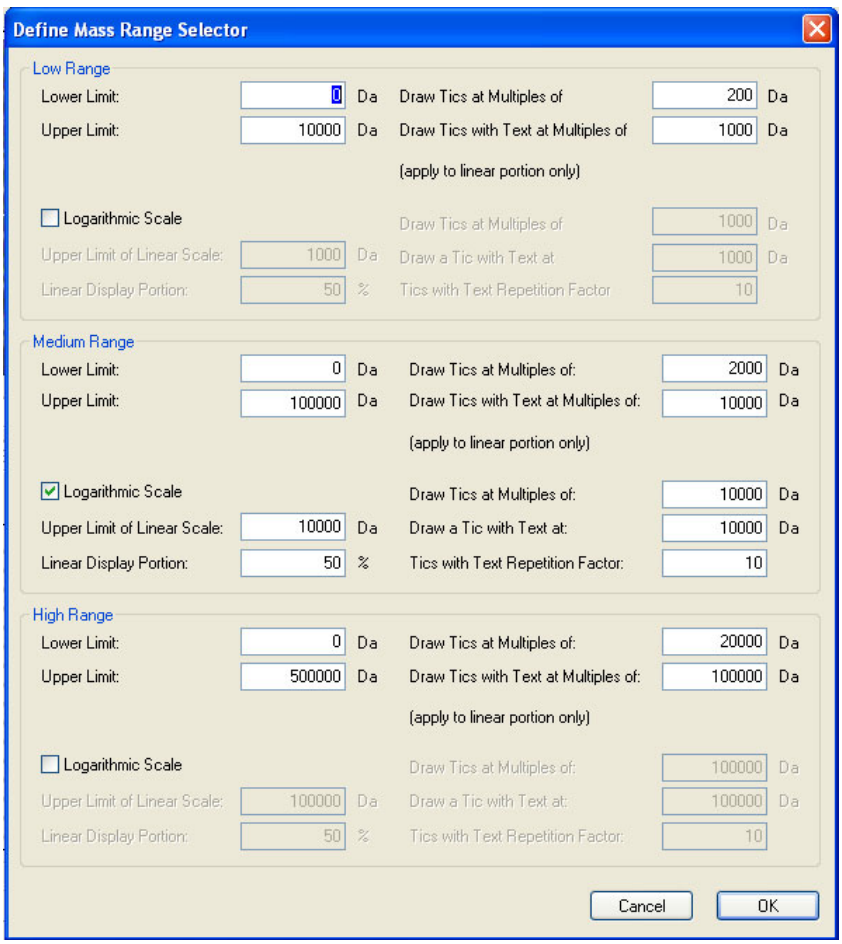
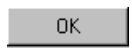

Define Mass Range Selector
Low Range
Lower Limit: 0 Da
Upper Limit: 10000 Da
Draw Tics at Multiples of 200 Da
Draw Tics with Text at Multiples of 1000 Da
(apply to linear portion only)
Logarithmic Scale
Upper Limit of Linear Scale: 1000 Da
Linear Display Portion: 50 %
Draw Tics at Multiples of 1000 Da
Draw a Tic with Text at 1000 Da
Tics with Text Repetition Factor 10
Medium Range
Lower Limit: 0 Da
Upper Limit: 100000 Da
Draw Tics at Multiples of: 2000 Da
Draw Tics with Text at Multiples of: 10000 Da
(apply to linear portion only)
Logarithmic Scale
Upper Limit of Linear Scale: 10000 Da
Linear Display Portion: 50 %
Draw Tics at Multiples of: 10000 Da
Draw a Tic with Text at: 10000 Da
Tics with Text Repetition Factor: 10
High Range
Lower Limit: 0 Da
Upper Limit: 500000 Da
Draw Tics at Multiples of: 20000 Da
Draw Tics with Text at Multiples of: 100000 Da
(apply to linear portion only)
Logarithmic Scale
Upper Limit of Linear Scale: 100000 Da
Linear Display Portion: 50 %
Draw Tics at Multiples of: 100000 Da
Draw a Tic with Text at: 100000 Da
Tics with Text Repetition Factor: 10
Cancel
OK

Figure 78 Configuring the mass ranges

The mass range selector dialog is used to configure any of the three mass ranges. Concerning the layout all the boxes correspond to each other. They contain identical functions.

To customize the range corresponding values have to be entered into the **Lower Limit** and **Upper Limit** fields. The beginning or end of the **Medium Range** can overlap the adjacent mass ranges. A mass range may be displayed as a whole, in linear mode, or logarithmic mode as well. A combination of both scales can be also performed at the users discretion by entering a value into the field **Upper Limit of Linear scale**. The value in **Linear Display Portion** defines how both scales share the range.

Scale marking of a range can be performed by setting values, where tics shall appear. Tics may be assigned with a mass value to enhance viewing. The repetition rate of this assignment is associated with the factor in the entry field **Tics with Text Repetition Factor** in the logarithmic portion.

To adopt all the new entries press the 

button.

3.4.9.8 Calibration (non-LIFT Method)

The Calibration dialog shown in Figure 79 appears in case of a non-LIFT method. The ***Calibration*** page is used to recalibrate the mass spectrometer. During the calibration, particular masses of the acquired mass spectrum are assigned to reference masses of the loaded reference list (depending on the Calibration strategy). Depending on the currently loaded method, **flexControl** offers different Calibration pages.

flexControl offers three calibration strategies:

- *Interactive* Calibration, automatic or manual, using calibration lists (MCLs).
- *Smart*, that is an easy-to-use calibration procedure for optimum results. The program reappraises the statistical frequency of occurrence of calibrants.
- *Statistical* calibration for peptide spectra.

Interactive Calibration

In the Interactive mode the operator can choose between automatic (

) or manual (click in the list) assignment of reference mass peaks. In both cases the peak detection parameter currently chosen in the Processing Parameter editor are used to create the peak annotations. It is recommended to use SNAP for mass ranges up to 6000Da and e.g. Centroid for higher mass ranges.

To assign the masses ***manually*** to the calibrants, click in the list and afterwards in the spectrum left to the corresponding peak. Masses are afterwards filled in the calibration list. You may use the scroll wheel of your mouse for further manual assignments.

The calibrant list shows names and masses of known substances, the corresponding masses found in the currently active spectrum, and the error in ppm and in Dalton. The error is re-calculated with each new assignment. The error in Dalton is calculated with $(current\ mass - reference\ mass)$, the error in ppm is calculated with $\left(\frac{current\ mass - reference\ mass}{reference\ mass}\right) * 10^6$.

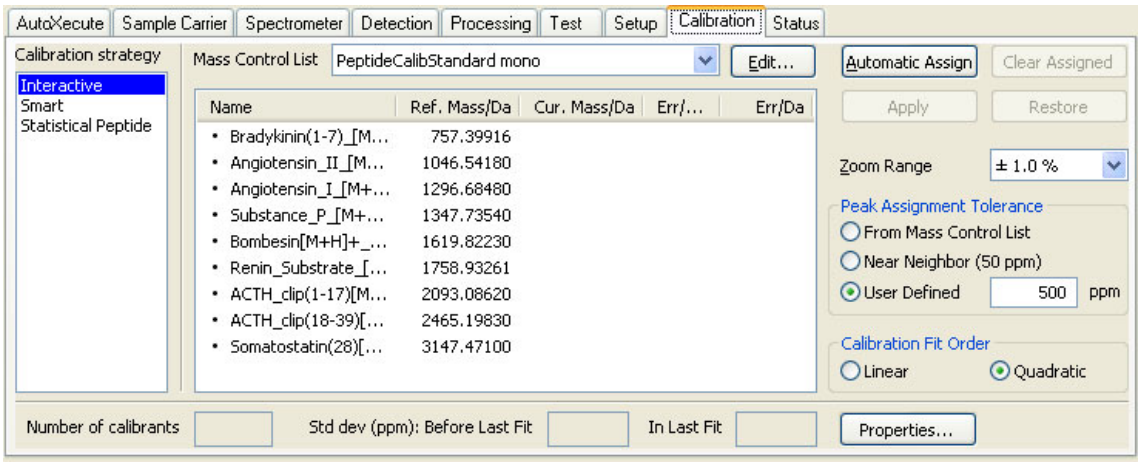


Figure 79 Features of the *Calibration* page

- Mass Control List Selection:

  This pull down menu contains the available mass control lists (MCLs). Only lists with at least one mass marked as calibrant are shown. The calibrants of the selected list are displayed in the box below.

- Button Edit...: Applying this button opens the Mass Control List Editor (Figure 80). MCLs can be changed and created from here, as well as from the AutoXecute Method Editor or from **flexAnalysis**. Pre-installed lists are write protected but can be used as templates for own user created lists. Whole peaklists (peaklist.xml) can be imported to use the masses as calibrants. For more detailed information on Mass Control Lists have a look in the *Quickstart_MassControlLists_Compass1_x.pdf* on the Compass CD.

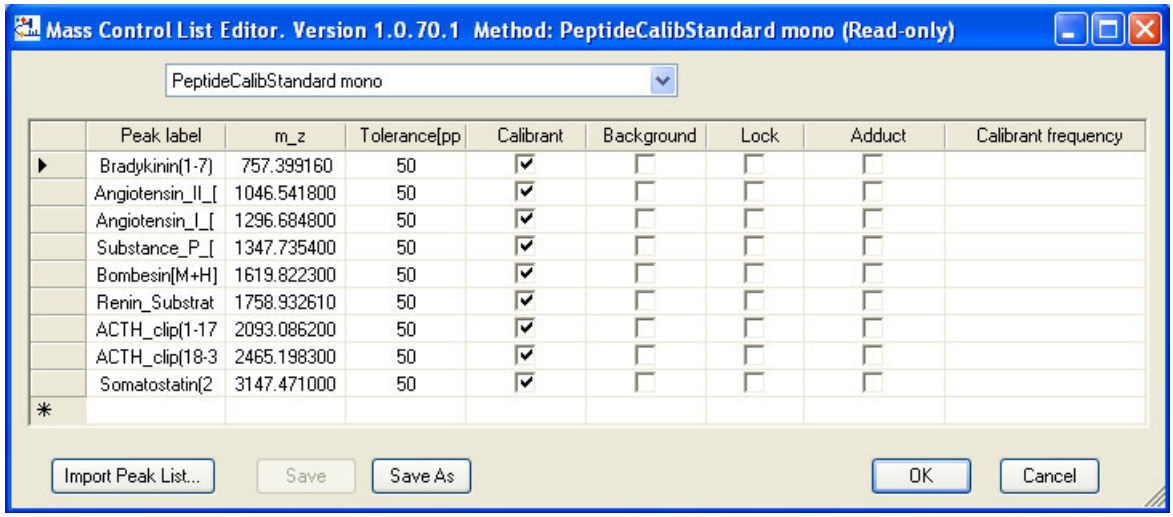


Figure 80 Mass Control List Editor

- Automatic Assign: Peaks from the spectrum are automatically assigned to the reference masses; a new fit result and errors are calculated but not yet applied to the spectrum. The peak finder algorithm used for automatic calculation is SNAP, if confirmed. The SNAP parameter can be adjusted via the Processing Parameter editor, but it is neither necessary nor recommended that SNAP is additionally selected as current peakfinder. Better select Centroid.
- Apply: With a click on this button the new fit result, calculated with the button “Automatic Assign” or manual assignment is applied to the spectrum (the spectrum is moved in the display).
- Restore: After the new fit result has been applied with “Apply” this can be cancelled with the “Undo” button. The original calibration, currently saved in the spectrum is applied again and peaks are re-annotated.
- Clear Assigned: This button deletes the assignments from the calibration list; it does not touch the possibly done calibration.
- Zoom Range off: For manual assignment of masses to reference masses it is necessary to click in the spectrum left to the peak of interest. Therefore it is helpful to zoom automatically the respective mass range corresponding to the selected reference mass. It is possible to choose between some relative and absolute zoom ranges.
- *Peak Assignment Tolerance*: The tolerance defines a mass interval that is used for the calibrant assignment. If the difference between a reference mass and a

peak in the peaklist is smaller than the tolerance, the peak is assigned to the calibrant. If more than one peak of the peak list fits this criterion the peak with the smallest mass difference to the calibrant is assigned to the calibrant.

- *Calibration Fit Order*: Selecting this option performs a quadratic or linear calibration calculating the coefficients c0, c1 (and c2). For linear calibrations c2 is set to zero.
- Properties...: The Properties dialog shows the current state of the calibration and some general calibration information. If spectra have been acquired with a **flexControl** HPC method the check box “High Precision Calibration applied” at the bottom (readonly check box) is activated.

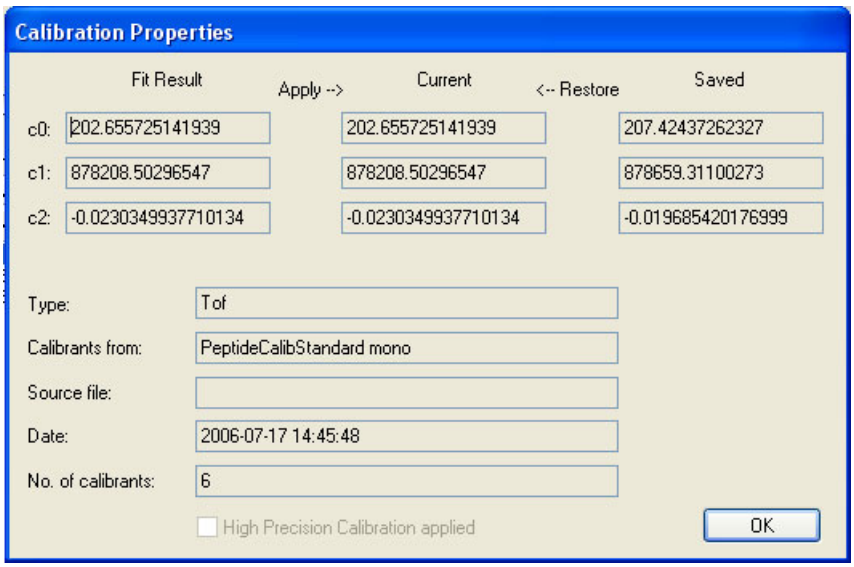
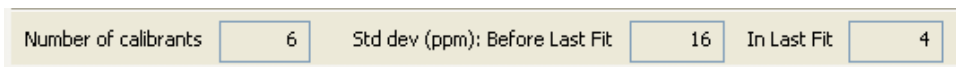


Figure 81 Properties dialog (same as in flexAnalysis)

- Result Information: Below the calibration list three result fields are shown: the number of used calibrants, the standard deviation before and standard deviation after the last calibration fit.



If no peaks are found during the peak assignment the following message appears.

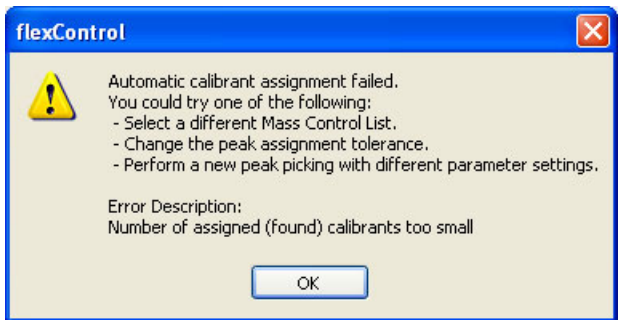

flexControl
Automatic calibrant assignment failed.
You could try one of the following:
- Select a different Mass Control List.
- Change the peak assignment tolerance.
- Perform a new peak picking with different parameter settings.
Error Description:
Number of assigned (found) calibrants too small
OK

Figure 82 Calibration failure

To redo the calibration perform the offered changes or open the Processing method and change for example S/N. Save and close the method an calibrate again.

Short workflow for the Manual Calibration

1. Acquire some spectra containing the masses, which are also calibrants in the selected **Mass Control List** and add the spectra to the sum buffer. The peaks must not be saturated!

2. Click the button Automatic Assign or select a Zooming Factor of for example 1% and mark with the left mouse button the first mass from the reference list, which is present in your sample. The selected mass is displayed as a red vertical line in the display. Click left to the peak that corresponds to the selected mass of the reference mass list. The line becomes green and the mass value is labeled at tip of the peak. The **Current Mass** and the **Error** is added to the corresponding column in the **Mass Control List**. The corresponding calibration constants C0 – C2 are displayed in the Properties dialog. Continue with the following masses by using the scroll function of your mouse. This has the advantage that you first may have a look on the actual labeled current mass and scroll then to the next (or the previous) reference mass. After assigning a mass, the corresponding row is marked with a check.

3.4.9.9 Calibration (LIFT Method)

If a LIFT method is loaded the Calibration page looks a shown in Figure 83. Calibration coefficients in LIFT applications are only valid over a small mass rage in contrast to coefficients in standard applications, which are valid over a wider mass range. Therefore LIFT applications need several sets of calibration coefficients to cover a wider mass range. It is only necessary to calibrate a LIFT method once, if no changes on the instrument took place. After the calibration it is not possible to change the method, because it is shown in a write protected “valid mode”.

Calibration coefficient sets are achieved by calibrating the fragment spectrum of one parent substance, called calibrant in this context. Collecting calibrants together with their associated calibration coefficients is performed by means of the ***Calibration*** page. This collection is stored in the method file. Thus, each LIFT method gets its particular calibration and spectra acquired with this method are automatically calibrated during the acquisition.

Calibrants additionally carry information concerning the applicability of their calibration coefficients. One part of this information is the mass interval for which this calibrant delivers useful calibration coefficients. The other part determines, whether the particular calibrant may currently be used to calibrate a LIFT spectrum.

The most prominent feature of the ***Calibration*** page is a list either displaying the calibrants of the currently loaded method, or the fragments of a particular calibrant, which are expected to be found in the spectrum. Buttons to handle calibrants are found at the bottom of the **Calibration** page. After one calibrant is selected and set as the LIFT parent mass (double-click or Set As Parent), its name and mass are displayed above the list. On the right of the ***Calibration*** page two groups of controls are shown. The group box 'Select Parent' is activated during the data acquisition and is used to acquire a whole LIFT spectrum of a particular calibrant, and to start calibrating it. The group box 'Select Fragment' is used to carry out the calibration process of the LIFT spectrum previously acquired, and is only activated after the data acquisition.

After loading a LIFT method the ***Calibration*** page appears in the so-called calibrants mode (Figure 83). This mode allows the user to edit the calibrant lists, select a calibrant for calibration, acquire a LIFT spectrum of this calibrant, and begin the calibration process, which will switch this page into the fragments mode.

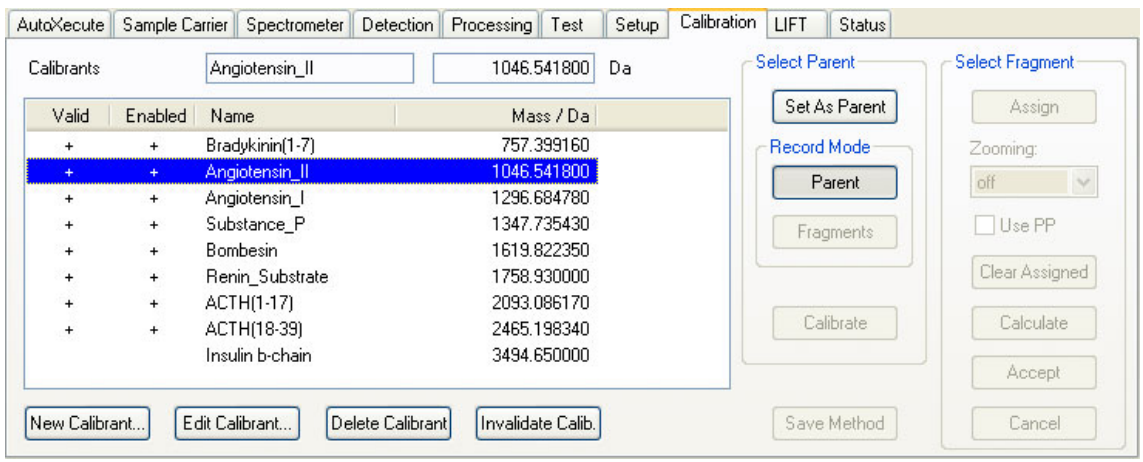


Figure 83 LIFT *Calibration* page in calibrants mode, valid method

In calibrants mode, the columns of the list reflect the properties of a calibrant.

- **Calibrants:** After a calibrant is set as parent mass, its name and mass are displayed here.
- **Valid:** A flag (+) indicates that the calibrant is in a well-calibrated state, i.e., no instrument parameter that influence the LIFT calibration has been changed since the last calibration process of this calibrant.
- **Enabled:** A flag (+) indicates that the operator allows using this calibrant.
- **Name:** This is the name of the calibrant. It does not affect the calibration or the LIFT measurement process.
- **Mass/Da:** This is the mass of the calibrant (parent ion).

The rows are listed in ascending order according to their mass.

When there is a calibrant selected and set as the LIFT parent mass, its name and mass are displayed in the fields above the list.

The four buttons at the bottom of Figure 83 are used for handling calibrants:

- New Calibrant...: Opens the **New Calibrant** dialog box (Figure 84) to enter the attributes of a new calibrant, such as name and mass.

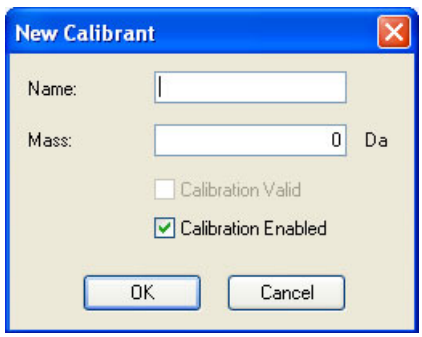


Figure 84 New Calibrant dialog

- Edit Calibrant...: Opens the **Edit Calibrant** dialog box to edit properties of the selected calibrant (Figure 85).

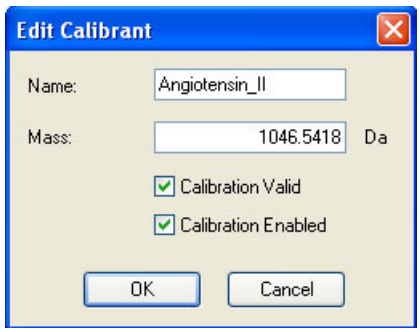


Figure 85 Edit Calibrant dialog box

- **Name**: An arbitrary string can be entered.
- **Mass**: No range checking is applied to the Mass. The user is responsible for a meaningful value.
- **Calibration Valid**: The user can invalidate a valid calibrant by clearing this checkbox. In contrast to this, an invalid calibrant cannot be made valid by selecting its "Calibration Valid" checkbox. It can only be made valid by calibrating it.
- **Calibration Enabled**: Clearing this box temporarily excludes a specific calibrant from a LIFT experiment.

- Delete Calibrant: Deletes the selected calibrant after confirming a warning. Before a calibrant will be deleted, the user must confirm this action in a dialog box (Figure 86).

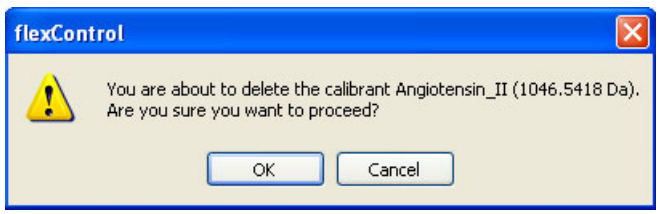


Figure 86 Confirm Deleting Calibrant

When the user confirms deleting, the calibrant will be completely removed. This deletion can only be undone by re-loading the method, discarding any changes since the last load.

- Invalidate Calib.: The state of all calibrants is set to invalid after the user has confirmed this in the dialog box shown in Figure 87.

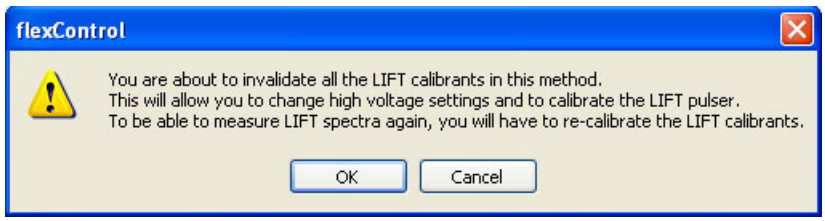

flexControl
You are about to invalidate all the LIFT calibrants in this method.
This will allow you to change high voltage settings and to calibrate the LIFT pulser.
To be able to measure LIFT spectra again, you will have to re-calibrate the LIFT calibrants.
OK
Cancel

Figure 87 Confirm Invalidation Dialog Box

The group box 'Select Parent' contains the following features:

- Set As Parent: The mass of the selected calibrant is set as the LIFT parent mass, i.e., the instrument is prepared to acquire a LIFT spectrum of this substance. The same can be achieved by double-clicking an item in the list. This button will only be active if one of the calibrants in the list is selected. When a calibrant has been set as parent, its name and mass will be displayed above the list.
- Parent: This button is active, if a calibrant is set as parent. In this mode the parent spectrum is acquired.
- Fragments: If this button is active the instrument is prepared to measure the fragment spectrum and the LIFT fine calibration is triggered. This button can only be accessed, if a (parent ion) spectrum is present in the sum buffer.
- Calibrate: Clicking this button initiates the actual calibration process by switching the ***Calibration*** page to fragments mode. This button is only active, if a LIFT spectrum is present in the sum buffer.

3.4.9.9.1 Fragments Mode

When the actual calibration process has been initiated, the page will be in fragments mode (Figure 88). The user can edit the list of fragments, assign peaks in the spectrum displayed to fragment reference masses, calculate a new calibration based on the assigned mass values, and accept or discard the newly calculated calibration, which will switch the page back to calibrants mode.

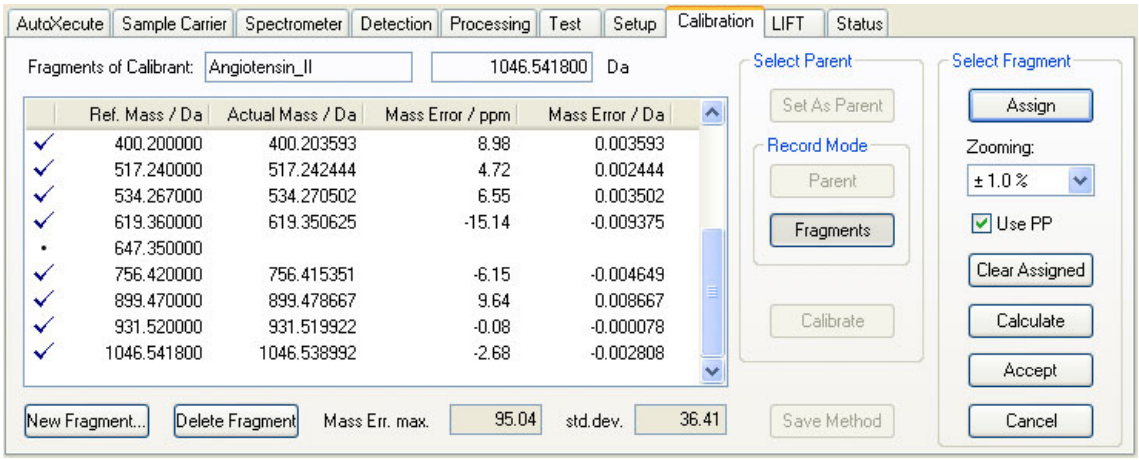


Figure 88 LIFT *Calibration* page in calibrate mode after fragment acquisition

- **Fragments of Calibrant**: Contains name and mass of the currently used calibrant to calibrate the instrument.
- **Status:** The symbol left hand side indicates the status of the fragment. A dot means that no actual mass is currently assigned to the particular fragment. An arrowhead indicates that an actual mass is currently being assigned to this fragment. When a mass assignment exists already for that fragment, the arrowhead is red otherwise it is green. A check mark means that an actual mass is assigned to the fragment.
- **Reference Mass:** This is the literature value of the fragment mass.
- **Actual Mass:** This is the mass of a peak in the spectrum displayed that has been assigned to the fragment.
- **Mass Error:** When a new calibration has been calculated, this columns display the difference between reference mass and actual mass in ppm and Dalton.

Two buttons below the list serve for housekeeping of fragments.

- New Fragment...: This button opens a dialog box to enter the mass of a new fragment.
- Delete Fragment: This button deletes the selected fragment, if the user confirms this action in an appropriate dialog box.

Two fields below the mass list display statistical information regarding the mass error when a new calibration has been calculated. These values help to assess the quality of the calibration.

The group box ‘Select Fragment’ contains control buttons used for the actual calibration process.

- Assign: Starts the assign procedure of actual masses to fragments. The button is pressed until assignment is finished. Assignment can either be finished explicitly by clicking the Assign button once more, or by clicking Calculate or Cancel. When during assignment the end of the list is reached, the procedure is also finished.

Alternatively masses can be assigned to fragments by double-clicking an item in the fragment list.

- **Zooming**: This drop down list facilitates peak assignment in the Spectrum Window. The zooming factor refers either to the respective reference mass, or to absolute mass units. Choosing "off" displays the whole mass range.
- **Use PP**: When this box is selected, click left of a peak in the display to assign it. The "Display Peak Finder" set in "Customize Online Processing" will be used to calculate the mass. It will appear as "Actual Mass" in the list. When the check box is cleared, the abscissa mass value of the point the user clicked in the spectrum display will be used directly.
- Clear Assigned: Clears all actual mass values and the mass error values. It can be used before starting a new assignment process.
- Calculate: A new calibration (i.e., a set of calibration coefficients) will be performed.
- Accept: Accepts the last calculated calibration and switches the ***Calibration*** page back to calibrants mode (Figure 83). For storing the updated calibration permanently, the method has to be saved. This can be achieved by clicking the Save Method... button.
- Cancel: Any calibration activities are discarded and the ***Calibration*** page is switched back to calibrants mode.

The button Save Method... is activated, when a calibration has been calculated and accepted. This function is the same as that of the command **`Save Method`** of the File Menu, or the corresponding feature button on the Toolbar.

3.4.9.9.2 New Fragment

A new fragment can be entered in the **New Fragment** dialog box (Figure 89). No range checking is applied to the entered value. The user is responsible for supplying a meaningful value.

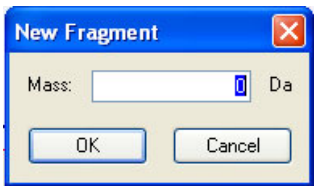


Figure 89 New Fragment Dialog Box

3.4.9.9.3 Delete Fragment

Before a fragment is deleted, the user must confirm this action in a dialog box.

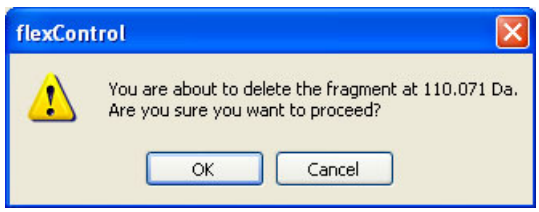


Figure 90 Confirm Deleting Fragment Dialog Box

When the user confirms deleting, the fragment is removed. This deletion can only be undone by re-loading the method, discarding any changes since the last load.

3.4.9.9.4 How to Use Buttons and Boxes to Calibrate the Instrument?

The aim of this section is to give an idea of the workflow. It cannot supply all the expert knowledge and experience needed to achieve an accurate LIFT calibration over a wide range of parent masses.

Initial conditions:

1. A target with the calibrant substance is introduced into the instrument.
2. The calibrants list contains masses of the used substance.
3. The instrument parameters of the loaded LIFT method are suitable to acquire good spectra.

At first, a LIFT spectrum has to be acquired:

1. Switch to the ***Calibration*** page.
2. If the list contains fragments rather than calibrants, click the Cancel button in the lower right corner.
3. Choose the first calibrant from the list used for the calibration, and double-click this calibrant in order to set it as LIFT parent mass.
4. Acquire a LIFT spectrum (parent and fragments) of the selected calibrant. The "better" the spectrum (low noise, high peak resolution, etc.), the easier is the calibration.
5. If the spectrum is satisfactory, start the calibration procedure with Calibrate.
6. Click Assign to assign the first fragment. The spectrum display will show a red line at the reference mass of the first fragment.
7. To assign the fragments click in the display left of the respective peak. The assigned mass will be entered in the list. Use the mouse wheel to switch to the next fragment. The position of the red line in the spectrum will then be updated to reflect the new fragment mass. If you want you can use peak picking (Use PP) for the peak assignment.
8. Repeat this until the end of the list is reached. Assignment will be automatically finished.
9. Click Calculate. Mass errors will be calculated and displayed in the list. The spectrum display will be updated to reflect the modified calibration. Note that at least 9 fragments have to be assigned to get access to the button Calculate.

Now it is time for fine-tuning the calibration. For this important part it is possible to delete single fragment assignments with a right-mouse-click that seem to be bad, or with the button Clear Assigned, to remove all actual masses from the list. Select a peak and click Assign to re-assign a different actual mass with it. After finishing click Calculate to update the calibration and the mass error display. If the calibration is acceptable, click Accept and save the updated calibration with Save Method....

3.4.9.10 LIFT

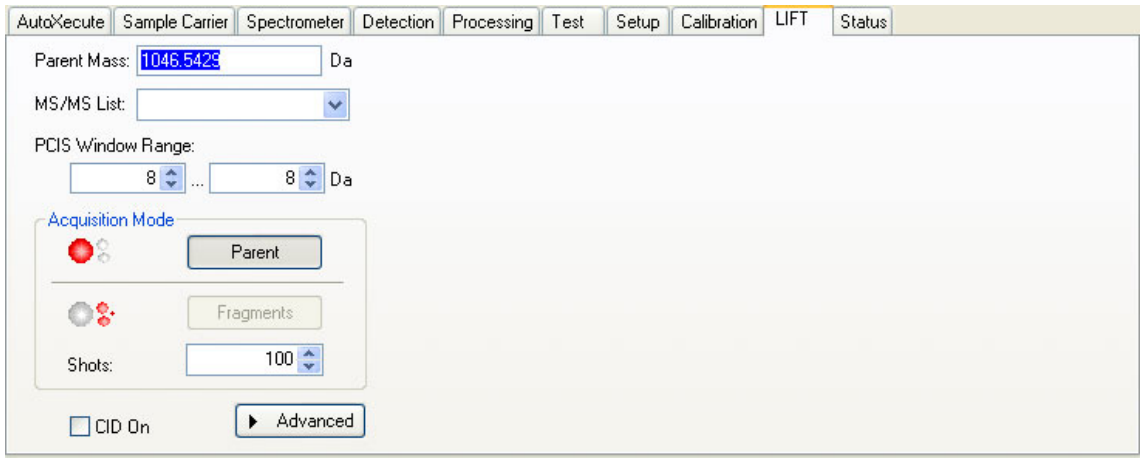


Figure 91 Features of the *LIFT* page

flexControl provides the ***LIFT*** page (Figure 91) in case of a tandem mass spectrometer and a loaded LIFT method (**<name>.**lft). This page is used to adjust and modify parameters during MS/MS experiments.

- **Parent Mass**: Just as in FAST-applications (section 3.4.9.11) here the parent mass has to be defined. Default is the mass, which has been entered at end of the last LIFT measurement or during the calibration in the ***Calibration*** page (Figure 83).
- **MS/MS List**: With this feature it is no longer necessary to type in (or copy and paste) the exact MS masses manually. The MS/MS list (Figure 92) can be filled with masses that have been selected in **flexAnalysis** from a MS spectrum and added there to the MS/MS list. The complete list can be sent to **flexControl** (for more details, have a look in the **flexAnalsis** manual). Additionally to the mass information the spot information where the MS spectrum has been acquired is shown, since an MS/MS spectrum can only be acquired from the spot where it's MS information comes from. If a mass from this list is selected the respective spot is automatically chosen (the target moves). After the LIFT spectrum is acquired and saved the mass is marked with x (Figure 92) in the list.

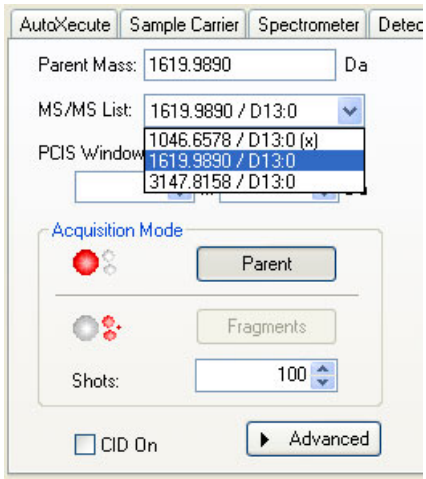


Figure 92 List with masses selected for MS/MS measurement

- **PCIS Window Range**: The borders of **PCIS Window Range** insulate the parent mass from other masses. The values preset in **Window Range** depend on the value set in **PCIS Range**. In this example the percentage of +/- 1% means that the window range of the current precursor mass (1046.54) is +/- 10 Da (refer to **PCIS Range** beneath).

- **Acquisition Mode**: On selecting the ***LIFT*** page the first time the Parent button is pressed. This button is the same as that in the ***Calibration*** page (Figure 83). The instrument is prepared to measure the parent ion spectrum.

  The Fragments button is also the same as that in the ***Calibration*** page. The instrument is prepared to measure the fragment spectrum and the LIFT fine calibration is triggered. The Fragments button can only be accessed when a parent ion spectrum is present in the sum buffer.

  Shots: Enter a number of shots for the **Fragments** measure mode.

- **CID On**: Checking this box opens a switching valve for argon to flow into the collision cell of the ion source.

  CID is an acronym for Collision Induced Dissociation. This term stands for the procedure where molecules get internal energy by collisions with gas molecules during a passage through a particular cell. The internal energy leads to so called metastable fragmentation, what is physically a spontaneous dissociation on a microsecond timescale. CID has proven to be useful to enhance intensities of fragments in the low fragment mass range and to differentiate between isobaric fragments like i/l peptide fragments.

The button Standard opens the group box 'Advanced Settings', which contains features to enable and recalibrate LIFT specific features. The accessibility of this group box depends on the *Bruker Daltonics UserManagement* given rights.

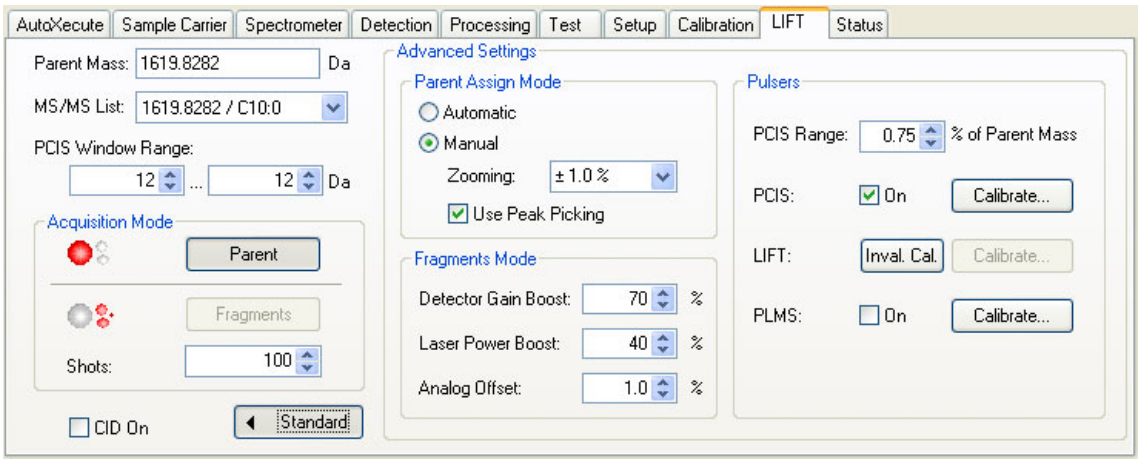
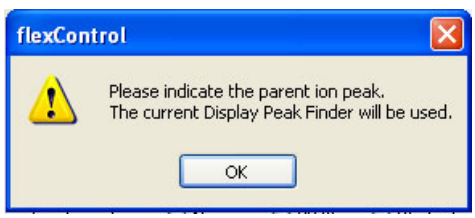


Figure 93 LIFT page – advanced mode

- **Parent Assign Mode**: This feature is used to assign the parent mass before switching to the fragments mode. If it is set to "Automatic" the parent mass is automatically detected, if it is set to "Manual" the parent mass has to be selected in the display (click left of the peak (with activated "Use Peak Picking")). The following dialog appears and must be confirmed with OK. **Note**: This feature applies only to manual LIFT acquisition. During the automatic run the parent is automatically detected, even if "Manual" is selected.



- **Fragment Mode**: **Detector Gain Boost** is used to enhance the appearance of fragments by increasing the detector gain. Defaults are loaded together with the LIFT method.

  Laser Power Boost is also used to enhance the appearance of small fragments now by increasing the laser power. Defaults are loaded together with the LIFT method.

  Analog Offset is used to raise the baseline of the digitizer. This may be useful to suppress the background noise of the electronic components, which are involved in guiding signals from the detector to the digitizer. As small fragments

may experience a boost in detector gain and laser power, an offset may be set without the risk disappearing in the noise.

- **PCIS Range**: The value entered here specifies the size of the window range around the precursor. The default of 1% should not be changed. But, if this value has been changed enter the precursor value a second time and press the **<Enter>** key. **flexControl** calculates the new window range.
- **PCIS**: The checkbox “On” is used to switch the precursor on or off. The button Calibrate opens the dialog **Precursor Ion Selector Calibration** (Figure 94).

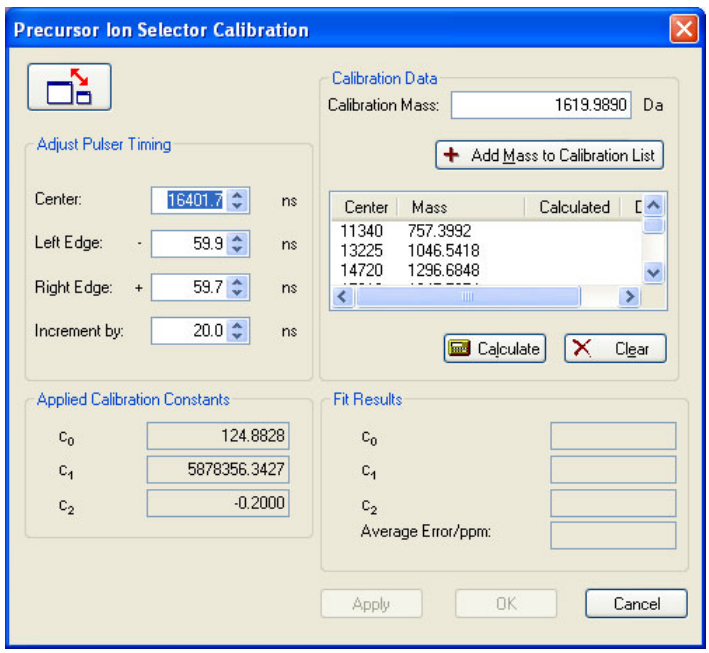


Figure 94 Ion selector calibration dialog

If the Ion Selector has to be calibrated for a specific mass type it in the field “Calibration mass”. The pulser timing will be calculated and has to be adjusted. If a well fitting timing is found appy the button Add Mass to Calibration List. The new calibration can now be calculated with the button Calculate and the constants are displayed in the field ‘Fit Results’. They are applied and transferred to the field ‘Applied Calibratio Constants’ with the button Apply.

The button top left on Figure 94 is used to reduce the size of this dialog.

- **LIFT**: There is no direct access to a LIFT calibration if you work with a validated LIFT method. The calibration can only be modified after invalidating the current calibration with the button Inval. Cal.. It is recommended to do a new calibration

only on the copy of an already calibrated and working method. For acknowledgement press the button “OK” on the corresponding warning message (Figure 95).

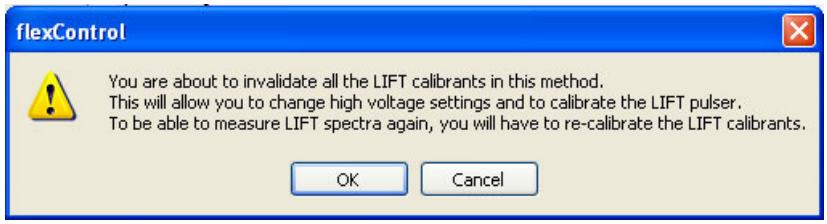

flexControl
You are about to invalidate all the LIFT calibrants in this method.
This will allow you to change high voltage settings and to calibrate the LIFT pulser.
To be able to measure LIFT spectra again, you will have to re-calibrate the LIFT calibrants.
OK
Cancel

Figure 95 Warning before invalidating a LIFT calibration

After that the related button Calibrate... becomes accessible to open the LIFT Pulser Calibration dialog (Figure 96).

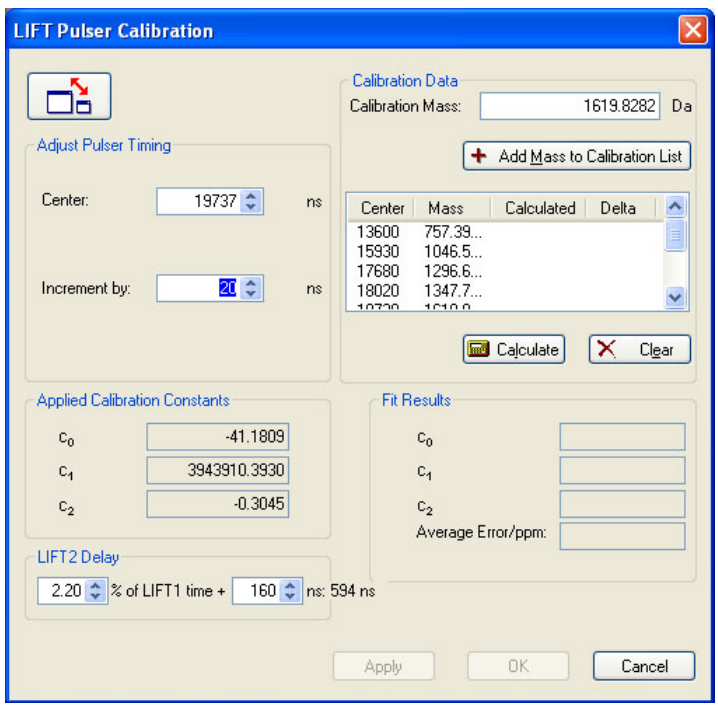


Figure 96 LIFT pulser calibration dialog

The usage of this dialog corresponds to that of the Precursor Ion Selector dialog (Figure 94).

- **PLMS** (**P**ost **L**ift **M**etastable **S**uppressor):
  To (de)activate the PLMS function manually use the corresponding check box in the group box 'Pulsers' (Figure 93). If the checkbox is selected, PLMS deflects parents formed after LIFT, together with the metastable fragments created after LIFT. As a result only desired fragments are visible. If the checkbox is de-selected, the LID fragments, the huge parent mass and metastable fragments of the parent mass created after LIFT (i.e., Post Lift Metastable fragment) are additionally visible.

  As a consequence MS/MS spectra are recorded in two steps:

  1. Checkbox PLMS = OFF together with low laser power: Basically the parent ion is detected, together with only very few LID and post LIFT metastable fragments. The signal recorded in this step is added to the sum buffer. A good resolved and clearly visible parent signal is desired for the later calibration of the MS/MS spectrum.
  2. Checkbox PLMS = ON: With increased (boosted) laser power and increased detector gain the fragments are recorded and also added to the sum buffer.

  These two steps are automatically performed during the manual and automatic acquisition of LIFT spectra.

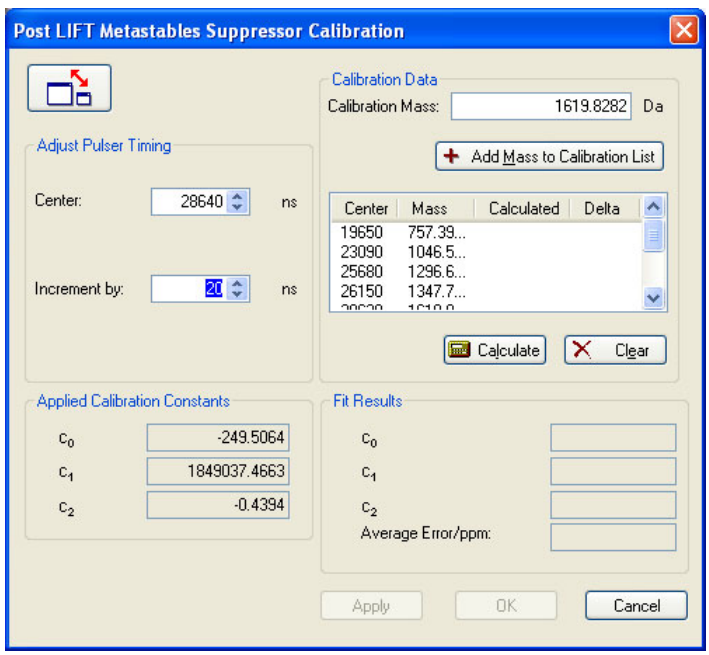


Figure 97 Post LIFT Metastables Suppressor Calibration dialog

3.4.9.11 FAST

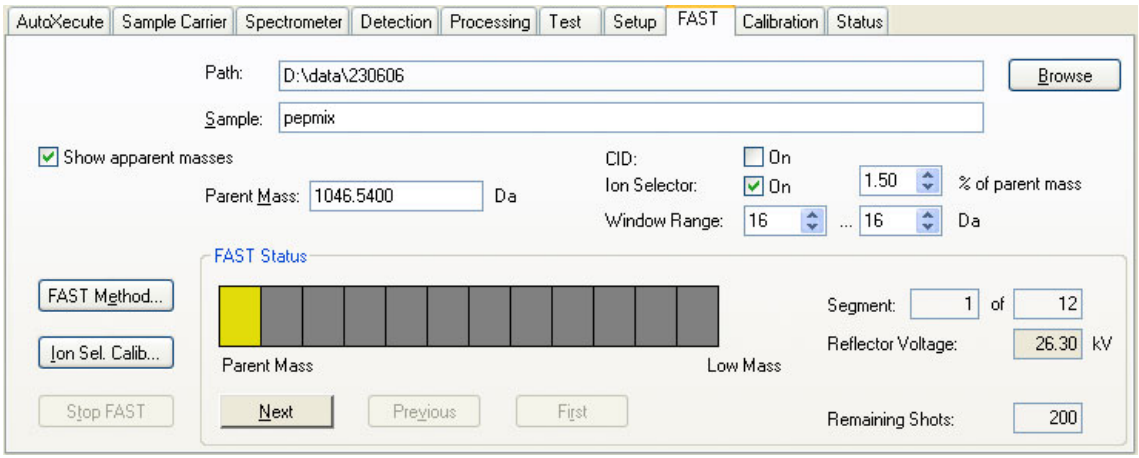


Figure 98 Features of the *FAST* page

Laser-desorbed ions may fragment during the flight in the field free region. Parent ions and fragments travel with the same velocity side by side through the TOF and strike the linear detector at the same time. A distinction of parents and daughter ions is not possible. Fragment spectra can only be obtained with a reflector instrument.

flexControl supports three ways to acquire fragment spectra:

1. Manual FAST measurement with reduced reflector potentials.
2. Automatic FAST measurement (***AutoXecute*** page) with reduced reflector potentials.
3. LIFT (***LIFT*** page), to record full fragment ion spectra within one single scan.

The ***FAST*** (**F**ragmentation **A**nalysis and **S**tructural **T**OF) page (Figure 98) appears automatically and only when a FAST-method (**<name>**.psm) is loaded. It is used to configure a FAST method for manual and automatic measurements.

During FAST measurements the reflector voltage is stepwise reduced to guide fragments to the reflector detector. Recorded spectra of the fragments can be finally combined to one linearized spectrum.

Compared with a standard acquisition method following parameters are additionally defined in a FAST method:

- Lowest fragment mass (lowest mass to be expected).
- Ion Selector Window settings (window isolating the parent mass).

Each of the following parameters is related to <u>one</u> segment exclusively:

- Total number of laser shots.
- Number of laser shots per sequence.
- Reflector detector gain.
- FAST high (upper limit of a FAST segment).
- FAST low (lower limit of a FAST segment).
- CID (**C**ollision **I**nduced **D**issociation).
- A FAST voltage list (reflector voltage for any FAST segment).

At the top of Figure 98 a path and a sample name have to be selected. Choose the path with the Browse-button, the sample name has to be typed in. The FAST files are stored in a folder like <path>\<Sample Name>\spot ID\experiment number\<parent mass>.FAST. If a folder of the same name already exists, the directory will be extended by a counter, e.g. experiment number**<parent mass>.FAST_2**.

After a path and a sample name are chosen, the parent mass of the FAST measurement has to be specified. To get only fragments of this ion it has to be isolated by checking the box “Ion Selector”. The mass range of the Ion Selector should be small enough to separate the parent mass from all the adjacent ions, which also deliver fragments.

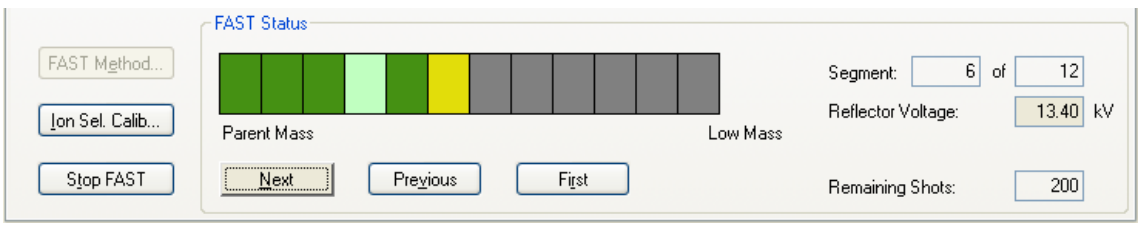


Figure 99 Group Box FAST Status

The FAST status indicator displays the current state of the manual FAST measurement. Any rectangle of the indicator symbols a FAST segment. The number of the displayed elements is variable and depends on the m/z ratio of the chosen parent mass (on the tab FAST) and the value of field **Lowest Fragment Mass** in the right upper corner of the FAST Method Editor (Figure 100). According to this relation the instrument provides up to 20 FAST segments (maximum).

To distinguish states of the FAST segments they are displayed in different colors. FAST segments, which are not yet processed, are grey. The FAST segment, which is just in progress, is colored yellow. FAST segments having been processed become green if the previously chosen total number of spectra is acquired and stored to the sum buffer.

The user can stop an acquisition at any time. In case of an earlier termination when the pre-selected maximal number of shots is not yet performed the field representing this FAST segment becomes light green in color.

Data storage is performed in a manner that the instrument first saves the shot sequences in the acquisition buffer. The user may add the contents to the sum buffer.

Right to the indicator elements is a group of two fields (view only) showing the number of the (yellow) **FAST segment** just being measured and the maximum number of elements referring to this measurement. The **Reflector Voltage** field beneath shows the reflector voltage of this active FAST segment. The view only field **Remaining Shots** displays a number of shots still left from the total number of shots previously set in the **Number of Shots for Segment** field of the **FAST Method Editor**. Any shot sequence added to the sum buffer reduces the contents of this display by the number of shots associated with this sequence.

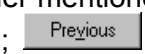
The three buttons

,

,

below the FAST segment indicator are used to navigate among the FAST segments for processing.

When applying the button

the first time the FAST folder mentioned above will be created. The adjacent element (*n*+1) becomes animated; 

turns to the element processed before (*n* -1). The button

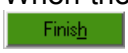
activates the most left FAST segment. The letter *n* represents the current FAST segment.

Re-selecting a FAST segment whose spectra are already stored to disk causes a reload of the data to the sum buffer and displays a spectrum in the related window.

Note: Reloading a spectrum sets the instrument in a state specified in the FAST method file! This state might be different to the spectrometer state associated with the reloaded spectrum!

Altering FAST segments for measurement causes in any case the storage of the spectra of the FAST segment just processed, erasing the acquisition buffer and sum buffer, and loading those parameters specific to the new FAST segment.

When the process of the last FAST segment is finished the

button toggles to 

and the spectrum is automatically saved.

- Checkbox “Show apparent masses” (Figure 98):

  If this box is activated, the RTD shows the mass range and accompanying peaks that are associated with the segment currently in use. Otherwise the entire mass range is displayed.

- Checkbox “CID”:

  Checking this box opens a valve for guiding collision gas into the collision cell of the ion source.

- Checkbox “Ion Selector”:

  This box is used to switch the Ion Selector on or off.

3.4.9.11.1 FAST Method Editor

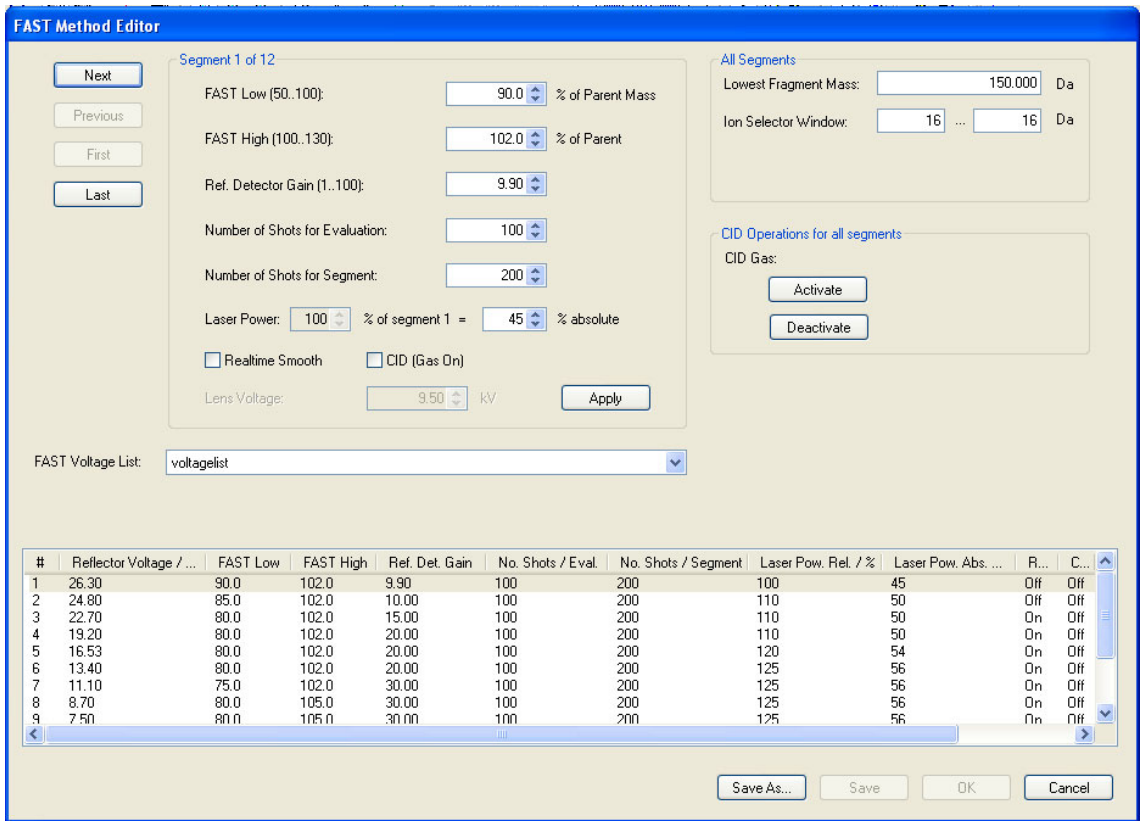


Figure 100 Features of the FAST Method Editor

On clicking the button FAST Method... on the dialog of Figure 98 the **Method Editor Window** (Figure 100) appears to modify or draft a FAST method.

The four buttons Next, Previous, First, and Last in the left upper corner are used to select rows of the currently loaded method to examine or modify parameters.

Each time on changing a FAST segment, parameters of the previous FAST segment are copied into the list of FAST segment specific parameters. The parameters of the newly activated FAST segment are displayed in the corresponding entry fields. The button Apply is related to the current FAST segment.

The group box ‘Segment 1 of 14’ requires FAST segment specific parameter inputs (The numbers 1 and 14 are variables and vary depending on the m/z ratio of parent and fragment masses as explained above).

The edit fields **FAST Low** and **FAST High** are used to specify the range of a FAST segment where the boundaries should overlap the adjacent segments. For the autoflex defaults are 80% of the parent mass for **FAST Low** and 102% of the parent mass for **FAST High**. Later on the segments can be joined together to a linearized spectrum. The **Ref. Detector Gain** depends on the S/N ratio.

The value edited in the field **Number of Shots for Evaluation** corresponds to the number of shot per sequence and is updated simultaneously in the field **Shots** of the **Target Manipulation Segment**.

The value edited in the field **Number of Shots for Segment** corresponds to the total number of shots, which are finally the contents of the sum buffer for this specific segment.

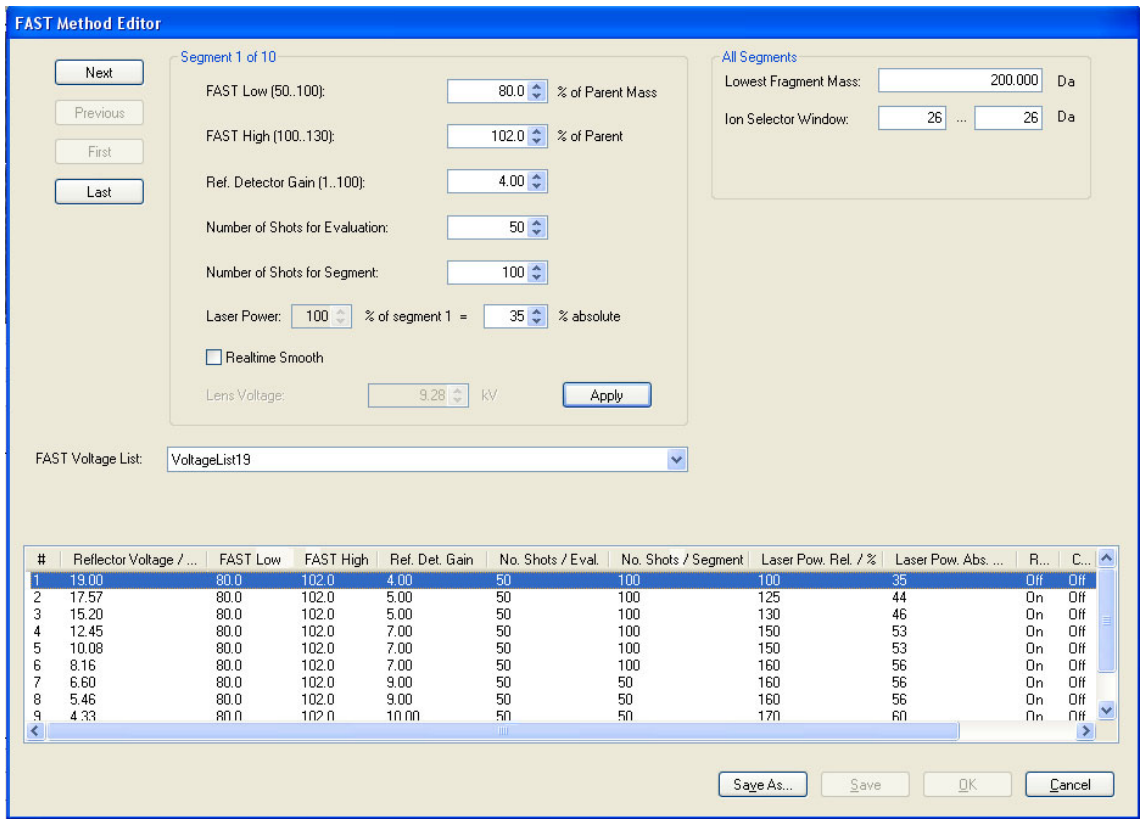

FAST Method Editor
Next
Previous
First
Last
Segment 1 of 10
FAST Low (50..100):
80.0
% of Parent Mass
FAST High (100..130):
102.0
% of Parent
Ref. Detector Gain (1..100):
4.00
Number of Shots for Evaluation:
50
Number of Shots for Segment:
100
Laser Power:
100
% of segment 1 =
35
% absolute
Realtime Smooth
Lens Voltage:
9.28
kV
Apply
All Segments
Lowest Fragment Mass:
200.000
Da
Ion Selector Window:
26
26
Da
FAST Voltage List:
VoltageList19
Reflector Voltage / ... FAST Low FAST High Ref. Det. Gain No. Shots / Eval. No. Shots / Segment Laser Pow. Rel. / % Laser Pow. Abs. ... R... C...
1 19.00 80.0 102.0 4.00 50 100 100 35 Off Off
2 17.57 80.0 102.0 5.00 50 100 125 44 On Off
3 15.20 80.0 102.0 5.00 50 100 130 46 On Off
4 12.45 80.0 102.0 7.00 50 100 150 53 On Off
5 10.08 80.0 102.0 7.00 50 100 150 53 On Off
6 8.16 80.0 102.0 7.00 50 100 160 56 On Off
7 6.60 80.0 102.0 9.00 50 50 160 56 On Off
8 5.46 80.0 102.0 9.00 50 50 160 56 On Off
9 4.33 80.0 102.0 10.00 50 50 170 60 On Off
Save As...
Save
OK
Cancel

Figure 101 The FAST Method Editor

flexControl is a universal control software for all the different mass spectrometers of the flex series. Taking into account the different instrument specific hardware, **flexControl** allows adjusting the total available laser power in proportional steps that is requested for the first segment of a specific instrument.
The function of the check box “Realtime Smooth” corresponds to that one in the ***Detection*** page (section 3.4.9.4). However, in this case it refers to one specific element.
The parameter *Lens Voltage* is the same as that in the ***Spectrometer*** page. If the CID function is selected the edit field in the dialog of Figure 101 becomes activated to set a value different to that previously set on ***Spectrometer*** (Figure 68).

The list contains all the editable parameters of the group box ‘Segment’ above and the previously selected voltage list. Any row corresponds to a voltage segment; the columns contain the segment specific adjustments. To experiment with parameters select a row that corresponds to the voltage segment you want to manipulate and edit the fields in the group box ‘Segment’. Any parameter change for a voltage segment takes effect with the button

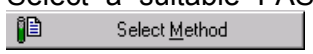
. For example, when Realtime Smooth is checked the corresponding position in the column **RTS** is set to **ON**.

The group box ‘All Segments’ contains parameters with a global character, which are therefore valid for the entire FAST spectrum. The first line is used to specify the **Lowest Fragment Mass,** whose relation to the parent mass corresponds to the displayed number of FAST segments on Figure 99.

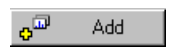
On clicking the

button the values are buffered and the dialog will be closed. The values are not stored in the FAST method file automatically.

3.4.9.11.2 Performing a Manual FAST Acquisition

1. Select a suitable FAST method (*.psm), if not yet loaded with the button 

on the **System Status Segment** of the GUI. The tab ***FAST*** immediately appears in the **System Configuration Segment**.
2. Start the acquisition by applying the button

on the **Acquisition Control Segment** of the GUI (Figure 37).
3. Add the acquired spectrum to the sum buffer by applying the 

button.
4. Repeat this action until the number of remaining shots in the corresponding field down right on the tab ***FAST*** has become zero. The

button becomes green in color.
5. Apply the button

. The color of the first segment on the tab ***FAST*** changes from yellow to deep green; the second segment becomes active and yellow in color.

6. Repeat from step 2 to step 5 until all segments are processed. If the operator is satisfied with a smaller number of added spectra as defined in the method he may press the button Next between two scans. The current voltage segment becomes light green and the color of following segment turns to yellow: it is now active.
7. When all segments have been processed, they all are colored deep or light green. The color of the button Finish changes from grey to deep green.

The button Stop FAST at the left lower corner of the ***FAST*** page becomes active after the first segment is completely processed. It may be used to terminate the FAST measurement in between. The message shown in Figure 102 appears.

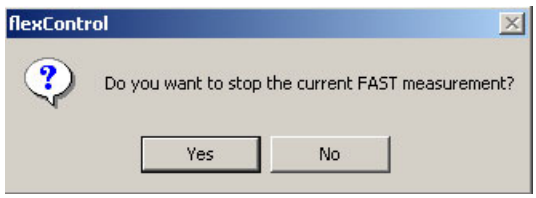


Figure 102 Apply "Yes" to terminate

A stop reloads instrument settings referring to the first FAST segment of the run.

3.4.9.12 Status Page

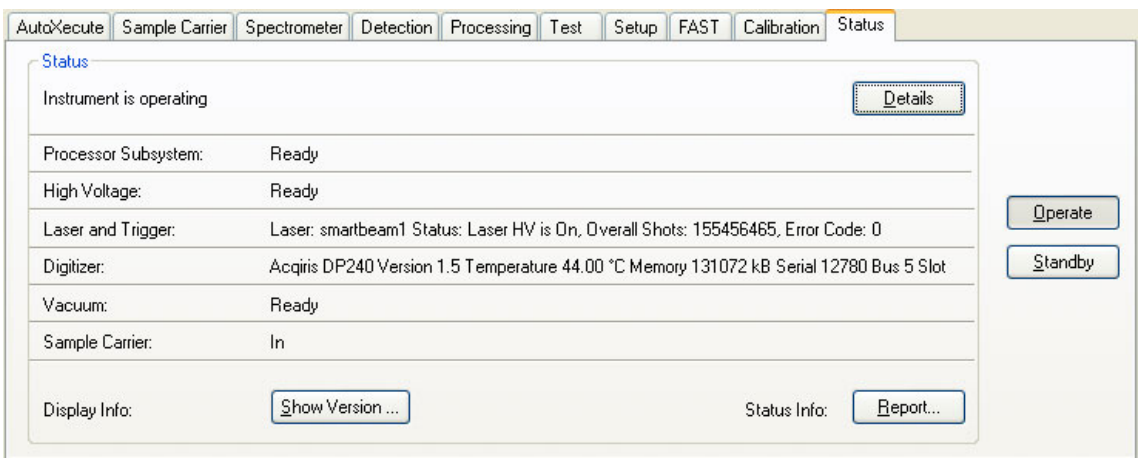


Figure 103 Features of the *Status* page

The ***Status*** page monitors the mass spectrometer and the attached peripheral equipment, such as the processor subsystem, high voltage, sample carrier, etc. Applying the Details button opens an additional set of pages reflecting detailed

instrument states. Here you can find e.g. the source cleaning maintenance interval bar, which gives you information about when you have to clean the source of your instrument by using the Source Shower target (Figure 104). As soon as 80% are reached, you will get the message, shown in Figure 105, which asks you to clean the source. For further information on using the Source Shower target, please refer to the Source Shower target manual.

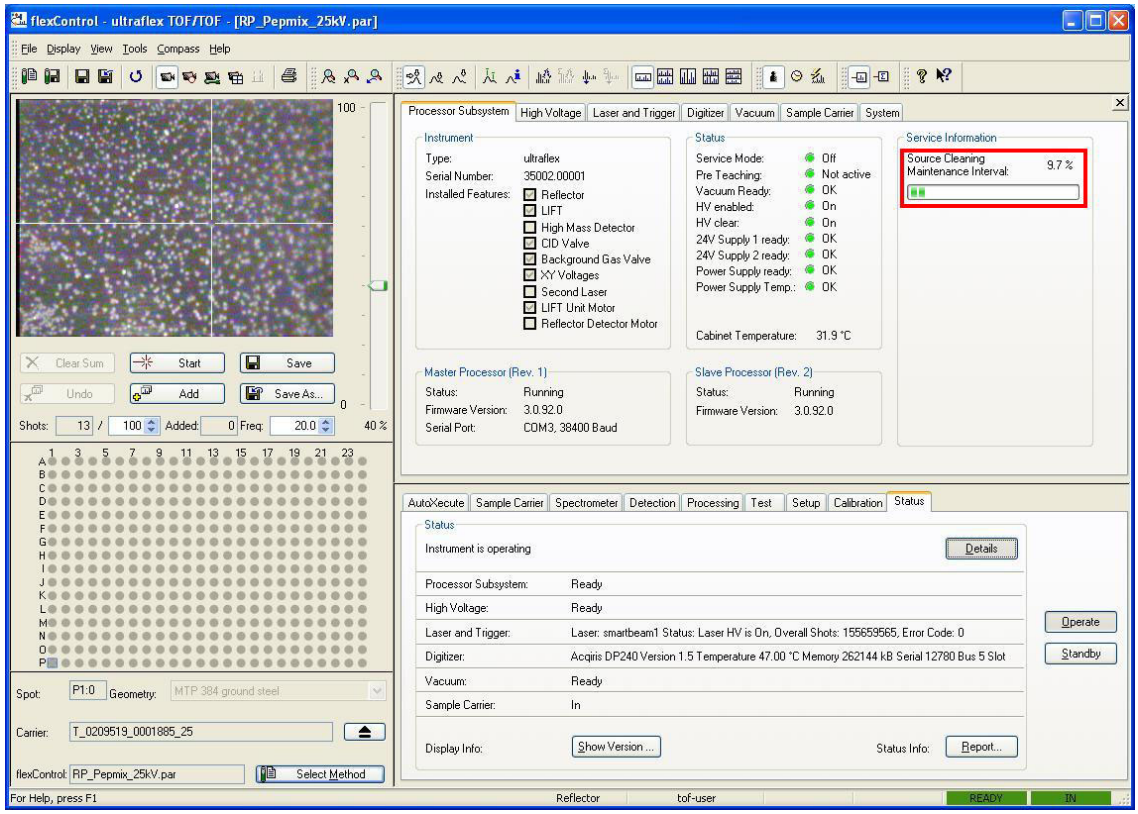


Figure 104 Status Details with Source Cleaning Maintenance Interval bar

TofControl
The source needs to be cleaned soon. Use the source shower target to perform the cleaning operation.
ErrorNumber:0x8F0000F6
OK

Figure 105 Source Cleaning Maintenance Interval >80%

On the right side of the status tab, there are the two buttons Operate and Standby to change the status of the mass spectrometer from the operation mode into the stand by mode and vice versa. These buttons are not available in case of a BIFLEX or REFLEX mass spectrometer.

Applying the Standby button right hand, the high voltage, the camera, the lamp, the laser, and the digitizer are switched off. Additionally following message appears:

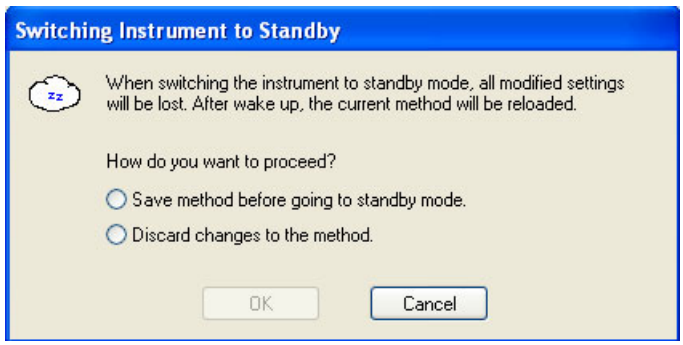

Switching Instrument to Standby
When switching the instrument to standby mode, all modified settings will be lost. After wake up, the current method will be reloaded.
How do you want to proceed?
Save method before going to standby mode.
Discard changes to the method.
OK
Cancel

Figure 106 Message asking how to continue

The Report... button opens the ***Status Reporter*** (Figure 107) that is used to collect helpful information of the instrument and the computer. The Status Reporter also starts in case of an error in **flexControl** to help the user to collect necessary information for the support concerning the current system state.

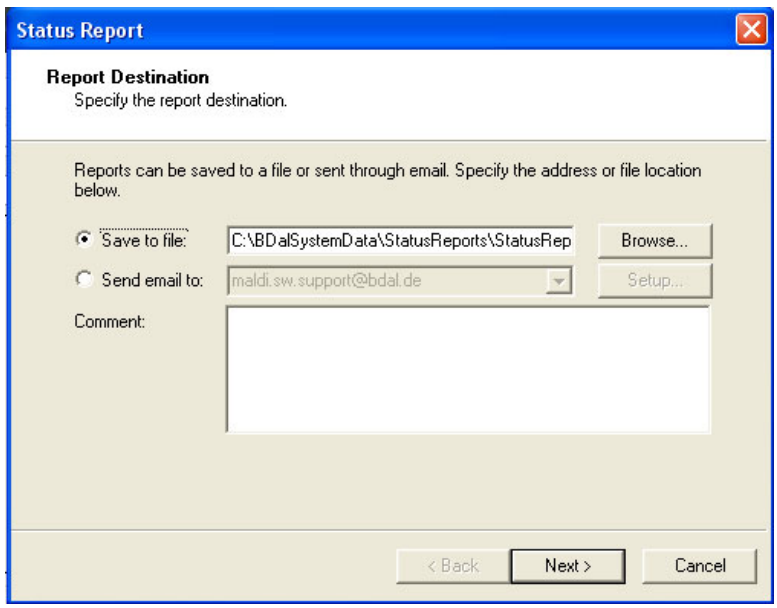


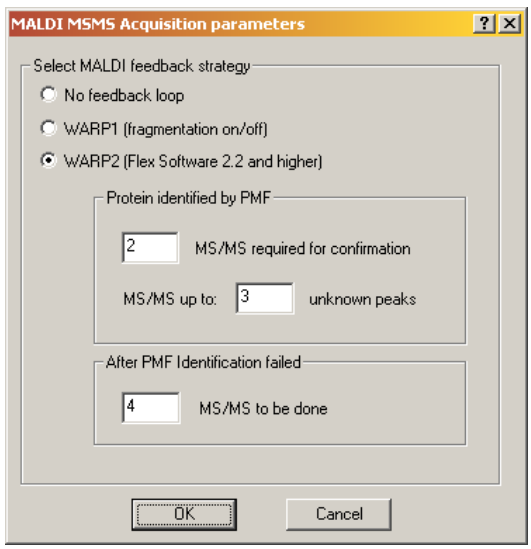
Figure 107 Status Reporter

A Appendix

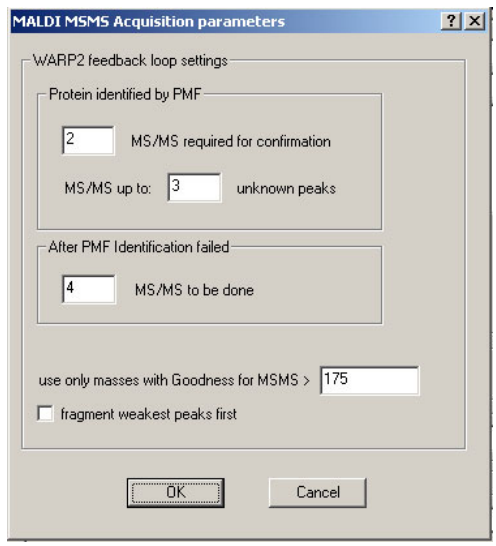
A-1 WARP feedback strategy with BioTools

The following workflow gives a short overview on the AutoXecute **WARP** feedback strategy that is used in combination with BioTools.

1. Select the WARP strategy *BioTools* in the AutoXecute settings dialog and re-start **flexControl**.
2. Setup an AutoXecute run with MS and MS/MS measurement.
3. Make sure that the MS spectra will be sent to BioTools after post processing in **flexAnalysis**, i.e. select a BioTools method in the AutoXecute MS method (Processing tab).
4. Make sure that the BioTools Feedback Parameters are selected correctly in the used BioTools MS method:



MS/MS acquisition in BioTools 3.0



MS/MS acquisition in BioTools 3.1

5. Make sure that **WARP** is selected in the AutoXecute MS/MS method. Otherwise the information BioTools adds to the peaklist will be ignored.

A-2 ISD

ISD spectra (**I**n **S**ource **D**ecay) are used to obtain sequence information directly from intact proteins. ISD MALDI spectra were introduced by R.S. Brown & J.J. Lennon Anal.Chem. **67** 3990-3999 (*1995*) and reflector ISD by Bruker: D. Suckau and D.S. Cornett Analusis **26 N°10**, M18-M21 (*1998*). In ISD spectra acquisitions, the molecular ions fragment spontaneously within the ion source and typically c- type fragment ions are formed (and possibly y-). The ions can be observed from the 10-15th N-terminal residue on and can cover large fractions of the whole protein sequence, provided that disulfide linkages were reduced and alcylated prior to ISD analysis. In reflector ISD, isotopic resolution and <0.05 Da mass errors can be expected in the range m/z 1500-3500, which allows to obtain sequence information close to the N-terminus, even in case of N-terminally blocked proteins not amenable to Edman sequencing. It appears to be a very useful method in the characterization of recombinant proteins.

Sample Preparation and Acquisition Protocol:

- *Preconditions:*

  Singly purified protein only (**no protein mixture!**).

  Sample at **10 to 20 pmol/µl** in 0.1 % TFA in water; < 20 % acetonitrile.

- *Material:*

  Matrix solution 1 (MS1): sinapinic acid (SA) saturated in ethanol.

  Matrix solution 2 (MS2): SA saturated in 0.1 % TFA, 30% acetonitrile.

- *Preparation:*

  Apply 1 µl of MS1 on stainless steel target to create a thin layer of matrix.

  Mix sample aliquot 1:1 with MS2 and apply 0.5 to 1 µl onto thin layer and let dry at ambient air.

  Prepare standard peptide mix for calibration (1000-4000 m/z) for reflector mode measurements. (Protein mixture for linear ISD with the goal of high sequence coverage up to larger masses.)

- *Parameter Settings:*

  Choose a parameter setting optimized for peptides using medium delay.

  Change digitizer sensitivity to 2 mV and set digital offset to 80 (Channel 1).

  Increase reflector detector voltage to 1.8-1.9 kV.

- *Acquisition:*

  Start analysis by collecting 50 shots each and increase laser power until peptide peaks appear.

  To obtain sufficient S/N ratio, several hundreds of shots must be added.

  Calibrate with standard peptide mix.

- *Processing in XMASS:*

  SNAP the region containing proper isotope-resolved peaks.

  Run the *Annotate Mass_Diffs* macro under *Aura_Cmds.*

  The sequences obtained here can be used for analysis; alternatively BioTools may be applied.

A-3 T^3-Sequencing

In source decay is a powerful MS/MS technique on the ultraflex, which allows to obtain fragment ion information near the termini from intact proteins. They must be pure and a few pmol must be present on the target.

ISD allows to obtain near-terminal sequence tags and acts as preparation technique for, e.g., c- and y-ions, which can then be MS/MS analyzed using LID or CID. As this technique enables “Terminal analysis by TOF/TOF” it has been termed T^3-sequencing.

Particularly c-ions are formed in ISD spectra, and sequence tags from monoisotopically resolved ions can stretch from 1-4 kDa, that allow for protein identification. These ions are equiintense independently of the particular sequence - a striking difference to classical CID-based MS/MS methods and prerequisite for the work with intact proteins.

Since c-type ions can be easily detected due to their intensity, they can be used for internal calibration, which achieves 5 ppm mass accuracy and facilitates the assignment of lower abundant a-, y- and z- ions and, therefore, the assignment of sequence tags from the C-terminus of the protein.

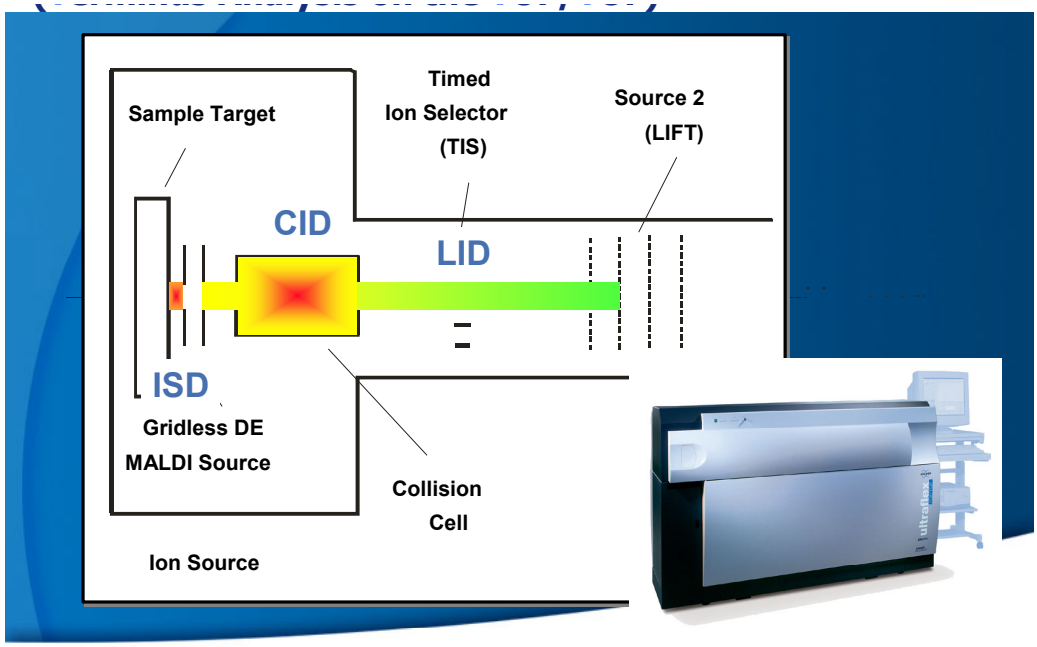


Figure 108 Terminus analysis on the TOF/TOF

Since ISD strictly occurs within the PIE ion source on a psec-nsec timescale and CID/LID strictly after the precursor ions have left the source, both fragmentations can be applied sequentially, essentially providing a pseudo-MS^3 technique.

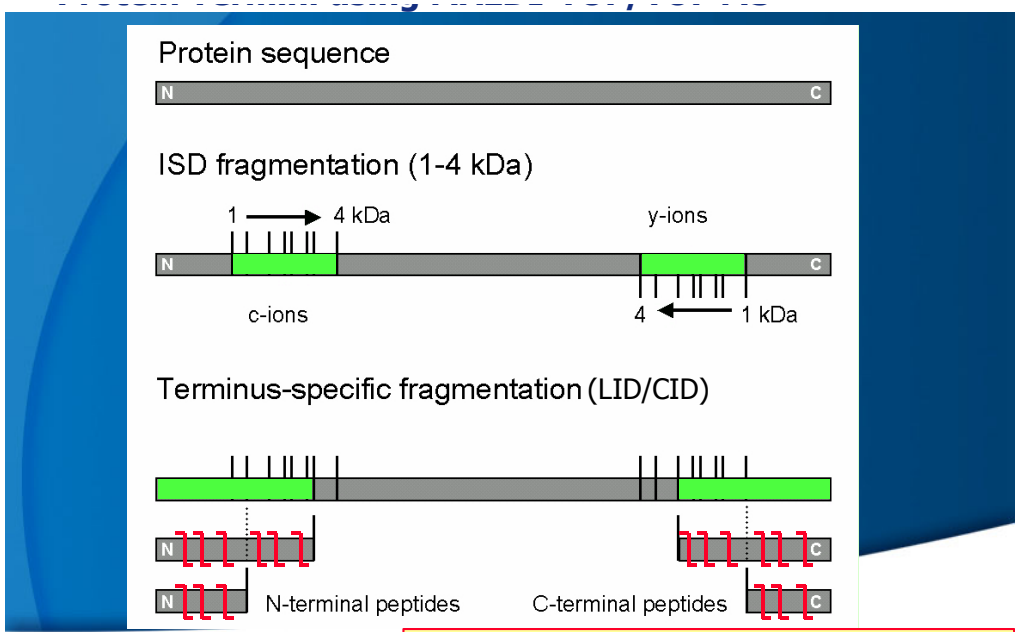


Figure 109 Protein Termini using MALDI_TOF/TOF MS

Fragment ion formation is a function of the selected matrix. While SA provides the cleanest c-ion readout and is well suited for reflector ISD measurements (reISD), DHB provides significant intensities of other ion types and is best suited for T^3-spectra acquisition.

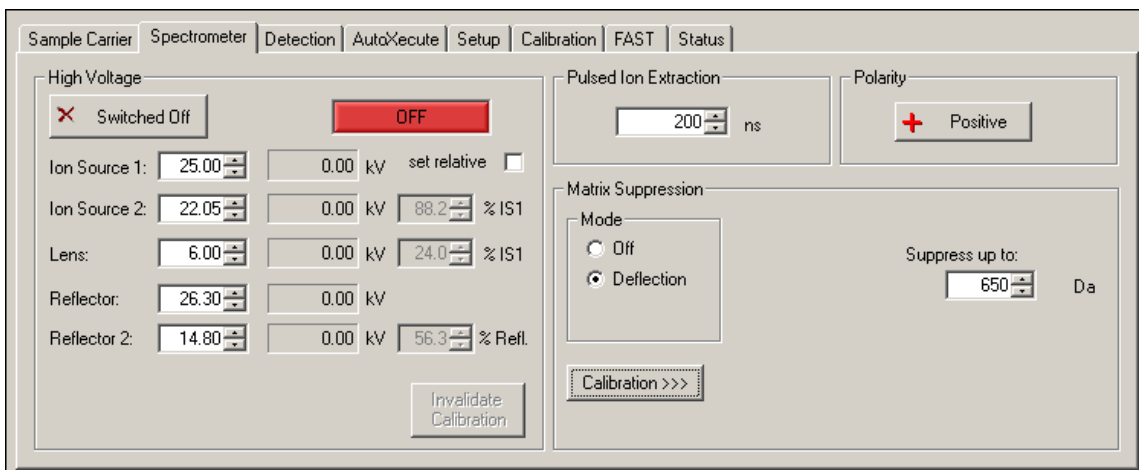


Figure 110 Typical ISD settings in the *Spectrometer* page

Start from a normal 25kV-Reflectron method and optimize resolution of ISD-fragments by tuning Ion Source 2 voltage and Pulsed Ion Extraction delay. Compared to peptide preparations in HCCA, DHB samples usually need higher IS2 and higher delay to achieve comparable resolution.

Low mass suppression is important and can be used up to 1000 Da.

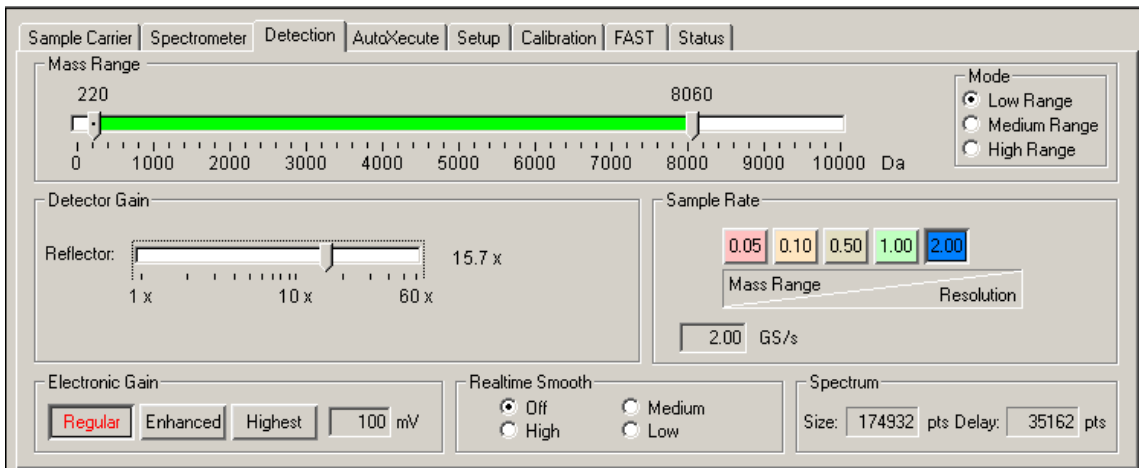


Figure 111 Typical ISD settings in the *Detection* page

Typical settings for detection gain: 15-40, depending on the sample.

Typical T3-Settings:

- Load the valid LIFT method.
- Type in the mass of an ISD fragment as parent. PCIS and PLMS settings are default.
- Take a LIFT spectrum the ISD fragment.
- High gain for reflector detector.
- 1000 laser shots or more.

Important precondition for T3-Sequencing: your TOF/TOF should be well tuned for Lift experiments. Especially Lift sensitivity is critical for T3-Sequencing experiments.

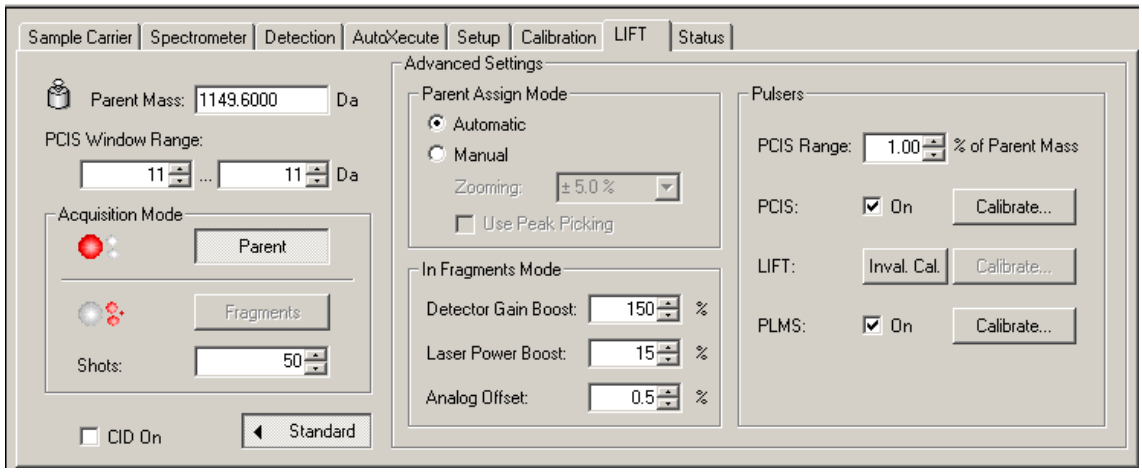


Figure 112 Typical T³ settings in the *LIFT* page

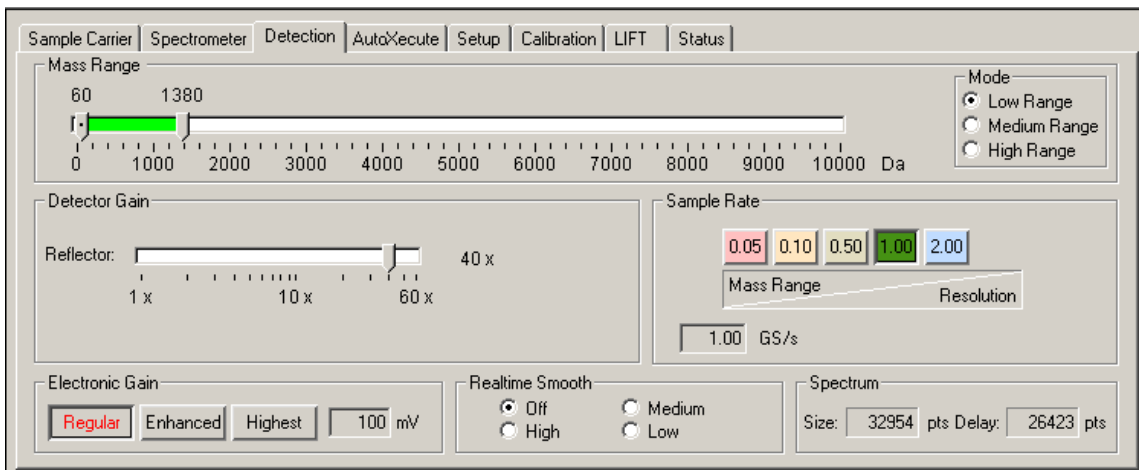


Figure 113 Typical T³ settings on the *Detection* page

Detection gain should be 25 or even higher. For spectrum judgement, smoothing (0.2 Da, 4 cycles) can be very helpful. Many hundreds of shots have to be acquired in fragment mode to get sufficient S/N.

A-4 Example of a Voltage List

An example of a section of a voltage list is shown in the example below. Any line corresponds to the reflector voltage [kV] of a segment.

```
20.00
16.00
13.11
10.61
8.59
6.95
```

A-5 Fuzzy Logic Control Module

What is Fuzzy Logic?

Fuzzy logic tries to resemble human reasoning. Inexact descriptions like “Somebody is small” or “The temperature is high” often leads to questions like “How many centimeters are small?” and “How many centigrades are high?”. For example, the fuzzy or multivalued logic approach allows any value on the temperature axis to represent the quality **high** to a certain degree. The degree is defined by a weight or truth function, which often falls down on both sides of a maximum and becomes zero outside a certain interval. Other temperature qualities, such as **low** or **medium** with overlapping truth functions may be added to build a fuzzy temperature description as shown in Figure 114.

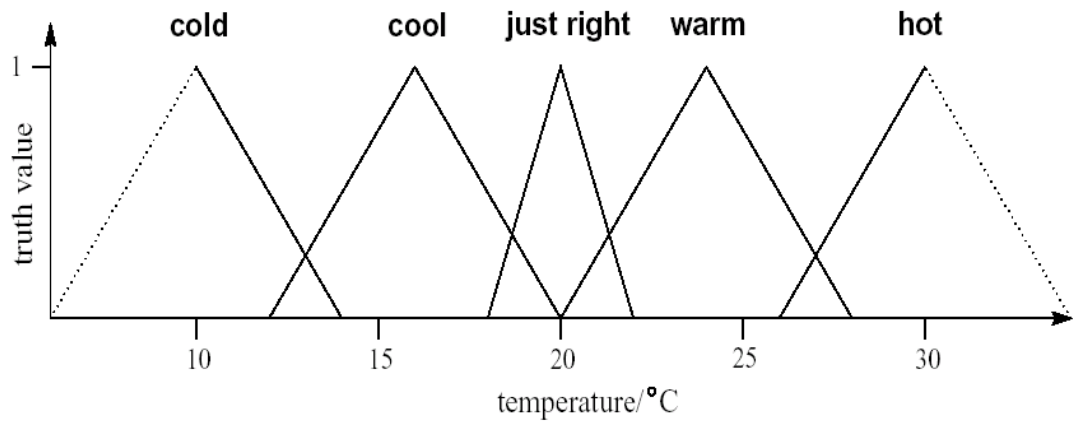


Figure 114 Overlapping temperature curves

The fuzzy temperature representation between 10°C and 30°C uses five slightly overlapping areas (fuzzy sets) describing five temperature qualities.

The main idea of fuzzy control lies in the usage of these overlapping sets covering the definition interval of each input and output parameter. A certain input value belongs to different input sets to a certain degree (fuzzification). Simple logical rules connect the input sets to appropriate output sets. For example, when the input value is a member of the set **I1** to a certain degree than the output value belongs to the set **O1** to this degree.

Finally a non-fuzzy output is retrieved from the output sets (defuzzyfication).

A simple example:

A simple example shall illustrate this. Suppose the goal is to build up a ventilator control subsystem for an air condition. The only input is the current room temperature. The only output is the relative speed of the ventilator system providing the room with fresh air. To simplify this example the heating of this room may be controlled by a different system. In order to achieve this goal with a fuzzy logic control system the input and output parameter space must be available in a fuzzy representation. For the temperature we can use the fuzzy representation of Figure 114. For the relative ventilator speed a straightforward approach may also consist of five fuzzy sets covering the speed parameter space (Figure 115).

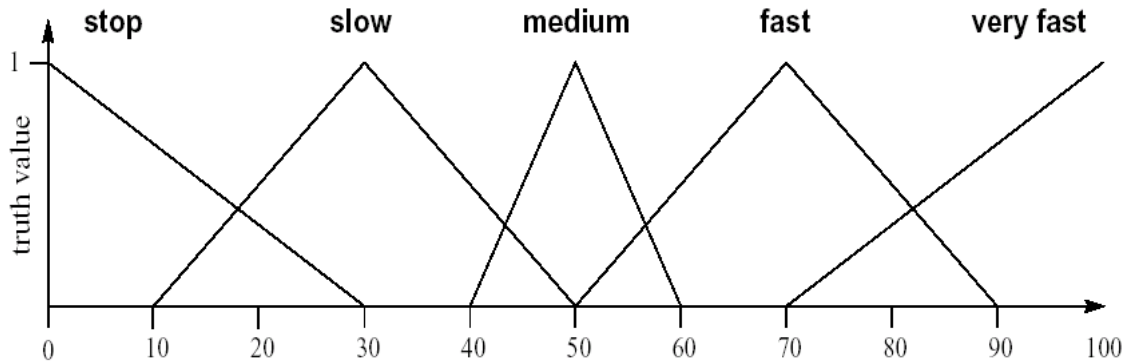


Figure 115 Relative ventilator speed

The fuzzy representation of the relative ventilator speed uses also five overlapping areas (fuzzy sets) for five different speed qualities. Now the fuzzy sets describing the temperature and the fuzzy sets describing the ventilator speed must be connected. This is done by several logical rules. If the input and output sets are given meaningful names the logical rules will describe the control system in a semantic meaningful way.

In our case we have the following five rules (In general the rules are more complicated employing more complex logical constructs.):

Rule 1: If temperature is **cold** then speed is **stop**.

Rule 2: If temperature is **cool** then speed is **slow**.

Rule 3: If temperature is **just right** then speed is **medium**.

Rule 4: If temperature is **warm** then speed is **fast**.

Rule 5: If temperature is **hot** then speed is very **fast**.

One evaluation cycle starting with a temperature of 21°C and resulting in a relative ventilator speed of 60.1 is discussed below (Figure 116).

- **Fuzzyfication step**: An input temperature of 21°C is presented to the system and is classified as belonging mainly to the temperature quality (fuzzy set) *just right* but also to a minor degree to *warm*.
- **Implication step**: According to the five rules stated above the *medium* quality is mainly activated (rule3) from the ventilator speed representation but also to a less degree the *fast* quality (rule4). The fuzzy output sets are scaled according to their activation.

- **Aggregation step**: All activated areas of the fuzzy ventilator representation are combined into a single fuzzy set in preparation of the final step.
- **Defuzzification step**: In order to gain just one number describing the new ventilator speed an average value must be somehow extracted from the fuzzy output. In our case a centroiding approach is used to achieve this. The result is 60.1.

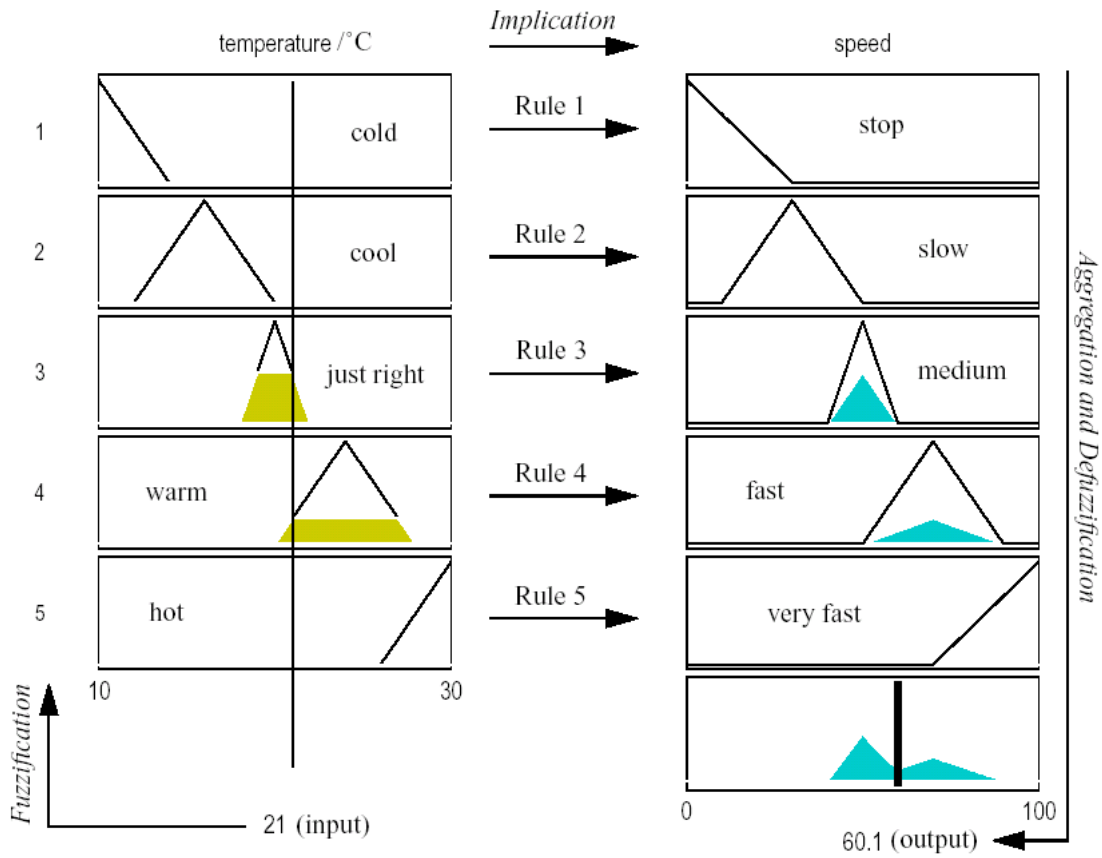


Figure 116 One evaluation cycle

One evaluation cycle for the fuzzy control logic based ventilator control system. The input value of 21°C is fuzzyfied. According to the specified implication rules some fuzzy output sets are activated to a certain amount. After having aggregated the activated output sets to one fuzzy output an averaging step extracts on specific output value (60.1).

To get an overall impression of the behavior of the controlling system an evaluation may be done for every input value, thus creating a temperature to speed mapping

(Figure 117). The result is a continuous piecewise linear function. It exhibits a very temperature sensitive behavior in the center part (small temperature changes lead to a large ventilator speed changes), surrounded on both sides by insensitive areas (flat curve parts), which prevent an overshooting of the controlling system. The flat curve parts at both ends of the temperature interval are due to the border effect and can be avoided by a more careful modeling. In order to get a smooth temperature to speed mapping the input fuzzification may be done using gaussian bell functions instead of triangles.

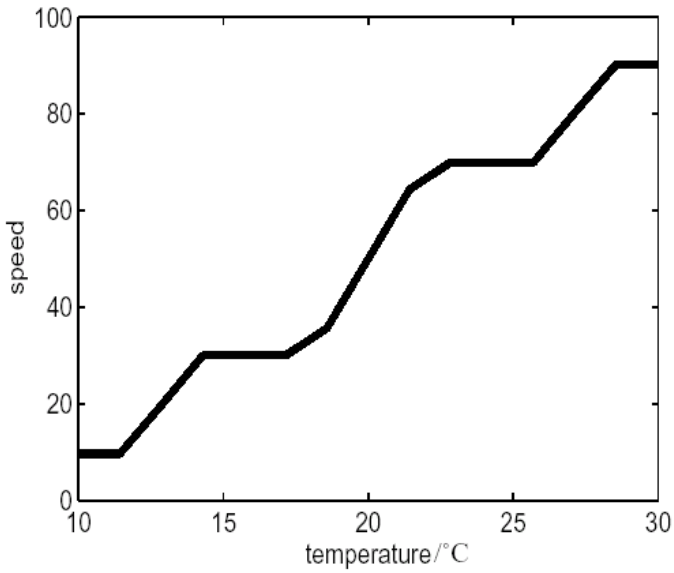


Figure 117 Temperature to speed mapping

Figure 117 shows the temperature to speed mapping resulting from the fuzzy logic based ventilator control system. The steep curve part in the center is responsible for a fast reaction on temperature changes around the optimal temperature value. The following flat curve parts provide insensitivity to small temperature changes there. The steep curve parts at the interval borders create a fast change of ventilator speed in order to leave these areas as soon as possible.

This example shows that even our simple approach to create a fuzzy representation of the input and output space together with five straightforward rules connecting input side to output side led to a well suited controlling system.

The Fuzzy Control of AutoXecute:

The fuzzy control module in **AutoXecute** has two input and two output parameters (Figure 118). It judges any acquired spectrum and decides whether the laser power

may be changed in order to increase the spectrum quality and if the spectrum is good enough to be added to the sum buffer, which will finally saved to disk. The fuzzy control module in **AutoXecute** takes intensity and resolution of the acquired spectrum to adapt the laser power and to decide whether data are good enough to be saved.

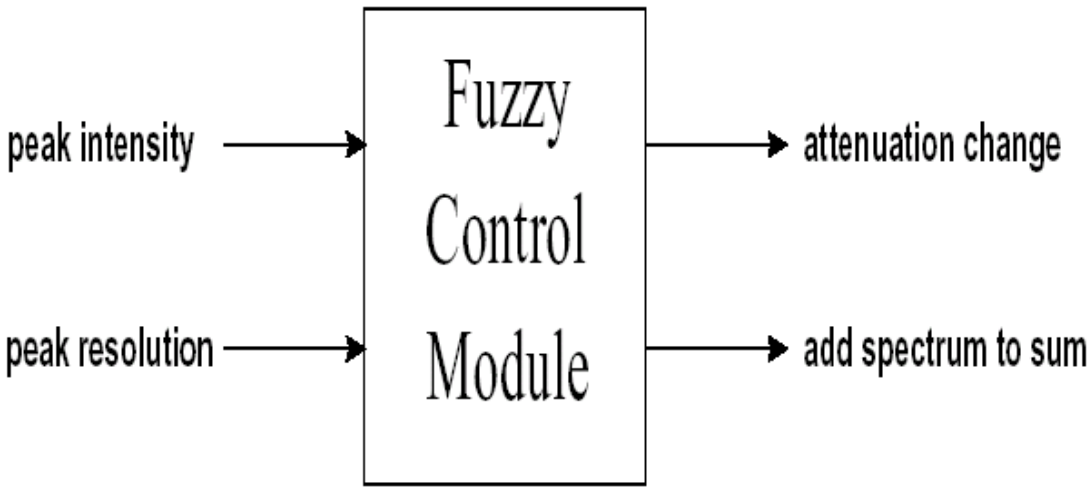


Figure 118 Scheme of the fuzzy control module

In order to cope with different substance groups and different instrument settings the fuzzy control module may be initialized with different fuzzy control files thus implementing special fuzzy engines, which define the acceptable intensity and resolution values for different measure processes. Currently are five fuzzy engines available (several more are presently tested and will be released soon).

Finally a 3D-plot (Figure 119) showing the rather non-linear connection between the two input parameters (intensity and resolution) and more input parameters (add spectrum to sum) may complete this section.

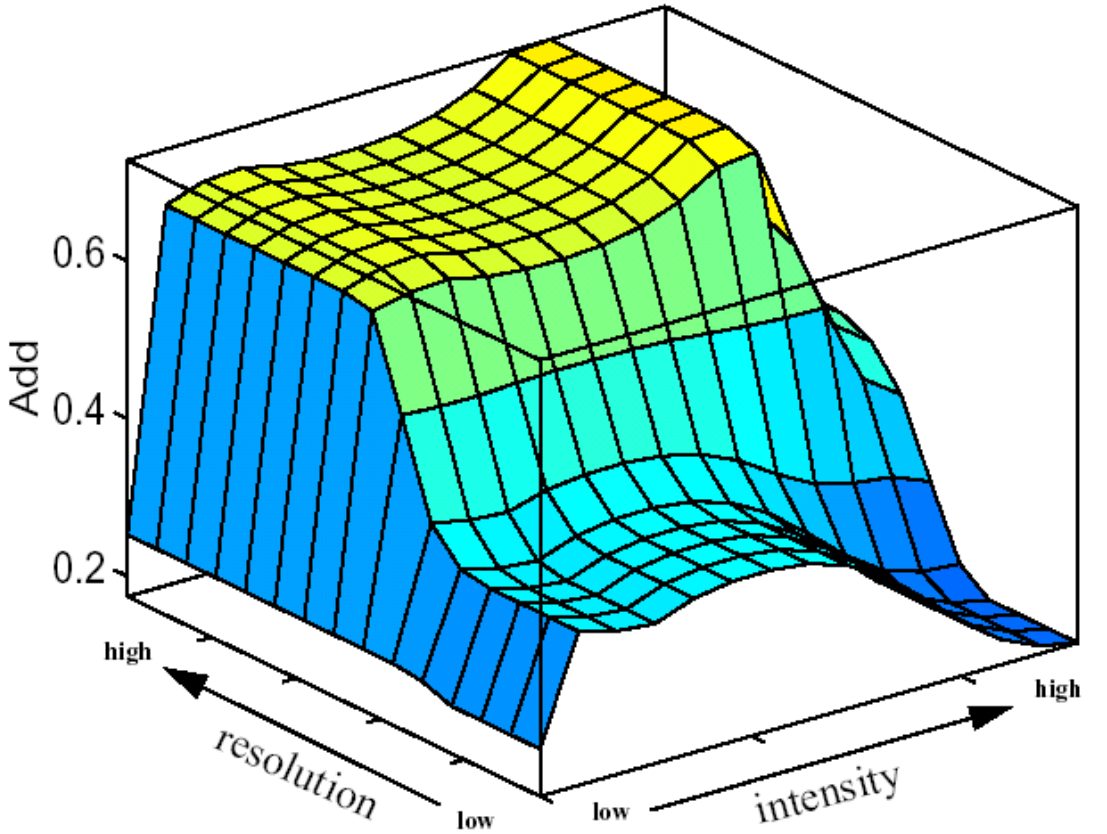


Figure 119 Dependency of the Add function from resolution and intensity

Only when the value of the adding output is big enough the sum is added to the sum buffer (higher area of the surface). Smaller values lead to a rejection of the spectrum (lower area of the surface).

List of Fuzzy Engines:

Fuzzy Engine	Description
peptides	The standard fuzzy engine for peptide measurements. (This fuzzy engine is identical to **tof_default**)
oligo_resx6	The standard fuzzy engine for oligo measurements. As difference to the *peptides* fuzzy engine *oligo_resx6* accepts very small per shot peak intensity. To avoid the summing of noise peaks, peak resolution **6** times the specified resolution threshold leads to a rejection of the spectrum (resolution too high).
oligo_resx10	This fuzzy engine behaves exactly as *oligo_resx6* except that too a high resolution is considered **10** times the specified resolution threshold.
oligo resx3	This fuzzy engine behaves exactly as *oligo_resx6* except that too a high resolution is considered **3** times the specified resolution threshold.
oligo resx2	This fuzzy engine behaves exactly as *oligo_resx6* except that too a high resolution is considered **2** times the specified resolution threshold.
fast_high_int	This is the fuzzy engine for automated FAST/PSD measurements on sample spots with relative high substance concentration.
fast_med_int	This is the fuzzy engine for automated FAST/PSD measurements on sample spots with medium substance concentration.
fast_low_int	This is the fuzzy engine for automated FAST/PSD measurements on sample spots with low substance concentration.

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